| |
|---|
| Mouri A, Noda Y, Shimizu S, Tsujimoto Y, Nabeshima T.<br>The role of Cyclophilin D in learning and memory. ***Hippocampus,*** 20. 293-304 (2010) 別刷 No.⑦ |
| Noda Y, Mouri A, Ando Y, Waki Y, Yamada S, Yoshimi A, Yamada K, Ozaki N, Wang D, Nabeshima T<br>Galantamine ameliorates the impairment of recognition memory in mice repeatedly treated with methamphetamine: involvement of allosteric potentiation of nicotinic acetylcholine receptors, dopaminergic-extracellular signal-regulated kinase 1/2 systems. ***Int J Neuropsychopharmacol.***, 11. 1-12 (2010) 別刷 No.⑧ |
| Mizoguchi H, Takuma K, Fukuzaki E, Ibi D, Someya E, Akazawa KH, Alkam T, Tsunekawa H, Mouri A, Noda Y, Nabeshima T, Yamada K.<br>Matrix metalloprotease-9 inhibition improves amyloid beta-mediated cognitive impairment and neurotoxicity in mice.<br>***J Pharmacol Exp Ther.*** 331. 14-22. (2009) 別刷 No.⑩ |
| Ibi D, Nagai T, Kitahara Y, Mizoguchi H, Koike H, Shiraki A, Takuma K, Kamei H, Noda Y, Nitta A, Nabeshima T, Yoneda Y, Yamada K<br>Neonatal polyI:C treatment in mice results in schizophrenia-like behavioral and neurochemical abnormalities in adulthood.<br>***Neurosci Res.*** 64. 297-305 (2009) 別刷 No.⑮ |
| Nagai T, Murai R, Matsui K, Kamei H, Noda Y, Furukawa F, Nabeshima T<br>Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D1 and serotonin 5-HT1A receptors. ***Psychopharmacology*** (Berl) , 202. 315-328. (2009) 別刷 No.⑱ |
| 野田幸裕　大橋美月<br>臨床に役立つ薬学研究の進歩　P-CUBE. 別刷 No.㉓ |
| 山田真之亮　野田幸裕　尾崎紀夫<br>神経発達障害・統合失調症関連遺伝子に基づく統合失調症モデルマウス，***モデル動物利用マニュアル.*** エル・アイ・シー. 印刷中 |
| 野田幸裕，毛利彰宏，鍋島俊隆<br>第1編　行動薬理研究における実験技術　10　統合失調症動物モデルとその評価法. ***実験薬理学シリーズ　第1巻,*** 印刷中 |
| 安藤　雄　野田幸裕　毛利彰宏　鍋島俊隆<br>統合失調症モデル動物に認められる行動異常***アニテックス***. 22, 20-25(2010) 別刷 No.㉒ |
| 野田幸裕　安藤　雄<br>精神科領域の薬剤開発－抗精神病薬の過去と未来－.<br>***薬事日報,*** 第 10704 号, p. 5 (2009) 別刷 No.⑳ |

野田幸裕
DS フォーラム(2009) 講演紹介 分科会 1 ドパミン関連セミナー ***臨床精神薬理***, 12, 160-169 (2009) 別刷 No.㉕

野田幸裕 毛利彰宏 脇由香里 鍋島俊隆
臨床知見に基づいた統合失調症動物モデルを作製するために-統合失調症モデル作成のため, 基礎研究者が臨床医に臨むこと-
***日本神経薬理学雑誌***, 29, 2 47-53 (2009) 別刷 No.㉔

**名古屋大学大学院医学系研究科・医学部附属病院薬剤部 教授** **山田清文**

Koike H, Ibi D, Mizoguchi H, Nagai T, Nitta A, Takuma K, Nabeshima T, Yoneda Y and Yamada K
Behavioral abnormality and pharmacologic response in social isolation-reared mice. ***Behav. Brain Res.*** 202. 114-121 (2009) 別刷No.②

Ibi D, Nagai T, Koike H, Kitahara Y, Mizoguchi H, Niwa M, Jaaro-Peled H, Nitta A, Yoneda Y, Nabeshima T, Sawa A, and Yamada K
Combined effect of neonatal immune activation and mutant DISC1 on phenotypic changes in adulthood. ***Behav. Brain Res.*** 206. 32-37 (2009) 別刷No.③

Arai, S., Takuma, K., Mizoguchi, H., Nagai, T., Kamei, H. and Yamada, K.
GABAB receptor agonist baclofen improve methamphetamine-induced cognitive deficit in mice. ***Eur. J. Pharmacol.*** 602. 101-104 (2009) 別刷No.⑥

Mizoguchi H, Takuma K, Fukuzaki E, Ibi D, Someya E, Akazawa K, Alkam T, Tsunekawa H, Mouri A, Noda Y, Nabeshima T and Yamada K
Matrix metalloprotease-9 inhibition improves amyloid b-mediated cognitive impairment and neurotoxicity in mice.
***J. Pharmacol. Exp. Ther.*** 331. 14-22 (2009) 別刷No.⑩

Ibi D, Nagai T, Kitahara Y, Mizoguchi H, Koike H, Shiraki A, Takuma K, Kamei H, Noda N, Nitta A, Nabeshima T, Yoneda Y and Yamada K
Neonatal polyI:C treatment in mice results in schizophrenia-like behavioral and neurochemical abnormalities in adulthood.
***Neurosci. Res.*** 64. 297-305 (2009) 別刷No.⑮

Nagai T, Kitahara Y, Shiraki A, Hikita T, Taya S, Kaibuchi K and Yamada K.
Dysfunction of dopamine release in the prefrontal cortex of dysbindin deficient sandy mice: an in vivo microdialysis study.
***Neurosci.Lett.*** 470. 134-138 (2009) 別刷No.⑭

Mizoguchi H, Arai S, Koike H, Ibi D, Kamei H, Nabeshima T, Kim HC, Takuma K and Yamada K
Therapeutic potential of nicotine for methamphetamine-induced impairment of sensorimotor gating: involvement of pallidotegmental neurons.
***Psychopharmacology.*** 207. 235-243(2009) 別刷No.⑲

| 名古屋大学大学院医学系研究科・<br>医学部附属病院薬剤部　　准教授 | 永井 拓 |
|---|---|

Nagai T, Kitahara Y, Shiraki A, Hikita T, Taya S, Kaibuchi K, Yamada K.
Dysfunction of dopamine release in the prefrontal cortex of dysbindin deficient sandy mice: an in vivo microdialysis study.
*Neurosci. Lett.* 470: 134-138 (2010)　　別刷No.⑭

Lu L, Mamiya T, Lu P, Niwa M, Mouri A, Zou LB, Nagai T, Hiramatsu M, Nabeshima T.
The long-lasting effects of cross-fostering on the emotional behavior in ICR mice.
*Behav. Brain Res.* 198. 172-178 (2009)　　別刷No.①

Koike H, Ibi D, Mizoguchi H, Nagai T, Nitta A, Takuma K, Nabeshima T, Yoneda Y, Yamada K.
Behavioral abnormality and pharmacologic response in social isolation-reared mice. *Behav. Brain Res.* 202: 114-121 (2009)　　別刷No.②

Ibi D, Nagai T, Koike H, Kitahara Y, Mizoguchi H, Niwa M, Jaaro-Peledf H, Nitta A, Yoneda Y, Nabeshima T, Sawa A, Yamada K.
Combined effect of neonatal immune activation and mutant DISC1 on phenotypic changes in adulthood. *Behav. Brain Res.* 206. 32-37 (2010)　　別刷No.③

Lu P, Mamiya T, Lu L, Mouri A, Niwa M, Kim HC, Zou LB, Nagai T, Yamada K, Ikejima T, Nabeshima T.
Silibinin attenuates cognitive deficits and decreases of dopamine and serotonin induced by repeated methamphetamine treatment. *Behav Brain Res.* 207. 387-393 (2010)　　別刷No.④

Lu P, Mamiya T, Lu L, Mouri A, Zou L, Nagai T, Hiramatsu M, Ikejima T, Nabeshima T.
Silibinin prevents amyloid beta peptide-induced memory impairment and oxidative stress in mice. *Br. J. Pharmacol.* 157, 1270-1277 (2009)　　別刷No.⑤

Arai S, Takuma K, Mizoguchi H, Ibi D, Nagai T, Kamei H, Kim HC, Yamada K.
GABAB receptor agonist baclofen improves methamphetamine-induced cognitive deficit in mice. *Eur. J. Pharmacol.* 602. 101-104 (2009)　　別刷No.⑥

Lu L, Mamiya T, Lu P, Toriumi K, Mouri A, Hiramatsu M, Kim HC, Zou LB, Nagai T, Nabeshima T.
Prenatal exposure to phencyclidine produces abnormal behaviour and NMDA receptor expression in postpubertal mice. *Int. J. Neuropsychopharmacol.* 19.1-13 (2009)　　別刷No.⑨

| |
|---|
| Lu P, Mamiya T, Lu L, Mouri A, Niwa M, Hiramatsu M, Zou L, Ikejima T, Nagai T, Nabeshima T.<br>Silibinin attenuates amyloid 25-35 peptide-induced memory impairments: Implication of inducible nitric oxide synthase (iNOS) and tumor necrosis factor-α (TNF-α) in mice. ***J. Pharmacol. Exp. Ther.*** 331 319-326 (2009) 別刷No.⑪ |
| Ibi D, Nagai T, Kitahara Y, Mizoguchi H, Koike H, Shiraki A, Takuma K, Kamei H, Noda Y, Nitta A, Nabeshima T, Yoneda Y, Yamada K.<br>Neonatal polyI:C treatment in mice results in schizophrenia-like behavioral and neurochemical abnormalities in adulthood.<br>***Neurosci. Res.*** 64. 297-305 (2009) 別刷No.⑭ |
| Nagai, T., Murai, R., Matsui, K., Kamei, H., Noda, Y., Furukawa, H. Nabeshima, T.<br>Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D1 and serotonin 5-HT1A receptors.<br>***Psychopharmacology***. 202: 315-328 (2009) 別刷No.⑱ |
| Takuma K, Fang F, Zhang W, Yan S, Fukuzaki E, Du H, Sosunov A, McKhann G, Funatsu Y, Nakamichi N, Nagai T, Mizoguchi H, Ibi D, Hori O, Ogawa S, Stern DM, Yamada K, Yan SS.<br>RAGE-mediated signaling contributes to intraneuronal transport of amyloid-β and neuronal dysfunction.<br>***Proc.Natl. Acad. Sci. USA*** 106. 20021-20026 (2009) 別刷No.⑰ |
| 永井拓, 鍋島俊隆, 山田清文. 統合失調症における発症脆弱性遺伝子と創薬.<br>***分子精神医学***, 9: 30-37 (2009) 別刷No.㉑ |

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| **小鹿一、間宮隆吉、鍋島俊隆** | **新規ステロイド誘導体及びその製造方法** | **特願 2009-039770** | **2010.2.22.** |

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Research report

# The long-lasting effects of cross-fostering on the emotional behavior in ICR mice

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ABSTRACT

Early-life stress during the postnatal period could precipitate long-lasting alterations in the functional properties underlying emotional expression in humans, but how the psychological stress of cross-fostering affects emotional behavior during adulthood in mice remains primarily unknown. The purpose of the present study was to examine the long-term effects of cross-fostering on the emotional behavior and cognitive functions of ICR offspring in adulthood. Cross-fostering was performed from postnatal day 7 for 3 weeks. Mice were divided into three groups: (1) biological group: pups born from ICR dams fostered by their original mothers; (2) in-foster group: pups born from ICR dams but adopted by other ICR dams and (3) cross-foster group: ICR pups adopted by C57 dams. ICR mice were subjected to behavioral experiments at the age of 8 weeks. Emotional behaviors in the cross-fostered mice were significantly altered in the open-field, elevated plus maze and forced swimming tests, as well as social interaction tests. However, the cross-fostered mice showed normal memory function in the Y-maze and novel object recognition tests. The contents of serotonin metabolisms were decreased in the prefrontal cortex and hippocampus indicated the deficit of serotoninergic neuronal function by cross-fostering. These findings suggested that the early-life stress of cross-fostering induced long-lasting emotional abnormalities, which might be possibly related to alterations of serotonin metabolisms.



## 1. Introduction

Stressful events have been implicated in the onset or exacerbation of psychological disturbances in humans [2]. Exposure to adverse events early in life, such as childhood neglect and physical or sexual abuse is regarded as one of the most prominent environmental factors associated with the increased risk of emotional disorders [13]. Evidence is mounting to support the hypothesis that adverse early environments underlie vulnerability to a variety of psychological disorders, such as anxiety, depression and schizophrenia [6,7].

Early-life stress, during the prenatal or postnatal period, exerts lasting effects on neural development thus affecting behavior in rodents [1,33]. For instance, rats exposed to prenatal stress in utero exhibit increases in anxiety or depression-like behaviors [20,36] and impaired cognitive function with aging [33]. Prenatal stress also induces long-term changes in neurobiological systems, including hyperactivity of the hypothalamo-pituitary-adrenal (HPA) axis in response to later stress [20]. Changes of postnatal interactions with pups and dams could-profoundly affect the emotionality as well as cognition of offspring in rats too [3]. It has been reported that maternal deprivation for long periods during the first 3 weeks of life impairs emotional behavior and affects pyramidal dendritic outgrowth in the prefrontal cortex [29]. In contrast, some postnatal manipulations have opposite effects on the development of offspring. Repeated maternal separation for a period of 15 min each day for 3 weeks, known as postnatal handling, has anxiolytic properties, which reduces anxiety-like behavior in adult rats [36], improves the performance of aged offspring in cognitive tasks [17], and attenuates stress-induced secretion of corticosterone.

Cross-fostering as a kind of postnatal psychological stress, could modify the mother–infants relationship early in life and mimic the psychology of childhood adoption which has frequently happened recently. Clinical researchers have reported that adopted children with a history of prenatal substance exposure [4,28] or placed relatively late in their adoptive home are at heightened

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risk of social, intellectual, and emotional problems [32]. Animal studies have also shown different responses of pups to adoption caused by differences in maternal care: BALB/cByJ dams displayed less nursing and licking/grooming of pups and spent less time in the nest than C57BL/6ByJ dams [10,11,30]. Other researchers have examined the influence of the interaction between genetic susceptibility and environmental factors on emotional behavior and reported that cross-fostering affects the level of anxiety in rats [12,39]. These findings indicate that cross-fostering may affect the behavior of offspring. However, most researchers have focused on the differences in maternal care between several species. It remains to be determined whether cross-fostering has long-lasting effects on emotionality as well as cognition in offspring during adulthood in ICR mice.

In the present study, to systematically investigate long-term effects of cross-fostering on emotional and cognitive functions in offspring, we examined emotional behavior, response to stress, social interaction, and cognitive function in adult ICR mice which had experienced cross-fostering for 3 weeks.

## 2. Materials and methods

### 2.1. Animals

Pregnant ICR and C57BL/6JJms Slc dams (E12) obtained from SLC Japan (Shizuoka, Japan) were maintained on a 12/12-h light/dark cycle (lights on from 08:00 to 20:00) with free access to food (CE2; Clea Japan Inc., Tokyo, Japan) and water. Dams were housed individually till parturition. ICR pups were weaned at 28 days of age and housed by sex in each group. Male pups were used for behavioral analyses at the age of 8–9 weeks. All of the behavioral experiments were carried out in a sound-attenuated and air-conditioned experimental room (23 ± 1 °C, 50 ± 5% humidity). The mice were habituated for at least 30 min before the tests and all behavioral tests were recorded by DVD camera to reconsider these results. The experiments were performed in accordance with the Guidelines for Animal Experiments of Meijo University and the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society (2007).

### 2.2. Cross-fostering procedure

Cross-fostering was performed at the postnatal day 7 (PD7) and completed within 5 min. Litters which consisted of 10 pups per dam with an equal number of males and females were used when possible. In this way, mice were divided into three different groups: (1) biological group: pups born from ICR dams and fostered by their original mothers; (2) in-foster group: pups born from ICR dams but adopted by other ICR dams and (3) cross-foster group: ICR pups adopted by C57 dams. All of the pups in each litter were taken out from their original cages and shortly separated from their original dams. Then, for the biological group, pups in the same litters were returned to their own dams one by one; for the in-foster group, the pups were put into their adopted dams within the same strain; while for the cross-fostered pups, they were put into other cages of C57 dams. All of the litters were composed of pups with same development history. Each group for each time contained more than three litters and were repeated more than 3 times. All of the pups were weighed once a week from birth to 8 weeks old. Male offspring were randomly used to check the behavioral changes and biochemical analyses.

### 2.3. Open-field test

As previously described [23], the apparatus for the open-field test consisted of a square area with black walls (*L* 40 cm × *W* 40 cm × *H* 40 cm) and was set in the experimental room. The floor of the field was divided into 64 identical squares (16 center squares, 16 corner squares and 32 other squares) so that the ambulation of mice could be measured. A light (100 W bulbs) was positioned 100 cm above the center. Each mouse was placed in the same corner square of the apparatus and allowed to explore freely for 5 min. During this period, the latency to cross the center squares, the time spent in the center and the corner squares, the numbers of ambulation, rearing and grooming events, and the frequency of defecation as well as urination were counted [30]. To count ambulation or rearing, entry into a square was defined as all four legs being inside the square. At the end of the test, the mouse was returned to its home cage and the apparatus was thoroughly cleaned with 70% ethanol.

### 2.4. Elevated plus maze test

The elevated plus maze was made of wood and consisted of two open arms (*L* 25 cm × *W* 8 cm) and two closed arms (*L* 25 cm × *W* 8 cm × *H* 20 cm) emanating from a common central platform (8 cm × 8 cm) to form a plus shape. The entire apparatus was elevated to a height of 50 cm above the floor. Testing commenced by placing a mouse on the central platform of the maze facing an open arm, and the standard 5-min test duration was employed. An entry was defined by all four legs entered into the arm. The open arm entries (%) and the time spent in open arms (%), and the total arm entries were calculated. After each test, the apparatus was thoroughly cleaned with 70% ethanol as previously described [40].

### 2.5. Forced swimming test

Mice were placed individually in a transparent polycarbonate cylinder (*φ* 8 cm × *H* 20 cm) containing water at 22 °C to a depth of 11.5 cm, and forced to swim for a 5-min period. The duration of immobility behavior was measured automatically by a SCANET MV-20 (Melquest Co. Ltd., Toyama, Japan), as described previously [27].

### 2.6. Social interaction test

The apparatus for the social interaction test was made of a gray polycarbonate (*L* 30 cm × *W* 25 cm × *H* 25 cm) [31]. Lighting in the experimental room consisted only of a dark light (25 W bulbs) and was diffused to minimize shadows in the arena. Before the test, each mouse was habituated alone in the apparatus for 10 min on two consecutive days. On the test day, the mice were randomly assigned according to gender to an unfamiliar partner in each group. The pairs of unfamiliar mice were placed in the apparatus for 10 min and the total amount of time spent in active social interaction, such as sniffing, grooming, following and mounting as well as crawling over or under the partner, was recorded. Passive contact (sitting or lying with bodies in contact) was not included in social interaction. At the end of the test, all the boluses were removed and the apparatus was cleaned with 70% ethanol.

### 2.7. Y-maze test

The Y-maze apparatus was made of wood and consisted of three arms (*L* 40 cm × *W* 12 cm × *H* 3 cm at bottom, *L* 40 cm × *W* 12 cm × *H* 10 cm at top) which converged at equal angles. Mice were placed at the center of the apparatus and allowed to move freely through the maze during the 8-min session. The series of arm entries was recorded visually. Alternation was defined as successive entry into the three arms on overlapping triplet sets. Alternative behavior (%) was calculated as the ratio of actual alternations to possible alternations (defined as the number of arm entries minus two) multiplied by 100 [25].

### 2.8. Novel object recognition test

The novel object recognition test was performed following previous reports [21]. The test procedure consisted of three sessions: habituation, training, and retention. Each mouse was individually habituated to the box (*L* 30 cm × *W* 30 cm × *H* 30 cm), with 10 min of exploration in the absence of objects for 3 days (habituation session). During the training session, two objects were placed in the back corner of the box. A mouse was then placed midway at the front of the box and the total time spent exploring the two objects was recorded for 10 min. During the retention session, animals were placed back into the same box 24 h after the training session, in which one of the familiar objects used during training was replaced with a novel object. The animals were then allowed to explore freely for 5 min and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counterbalanced manner in terms of their physical complexity and emotional neutrality. A preference index, the ratio of time spent exploring either of the two objects (training session) or the novel object (retention session) over the total amount of time spent exploring both the objects, was used to assess cognitive function.

### 2.9. Determination of monoamine and its metabolite levels in the brain

The prefrontal cortex and hippocampus were dissected out from the brains on an ice-cold plate immediately after the mice were decapitated. Each part of brain sample was quickly frozen and stored in a deep freezer at −80 °C until assayed. The contents of norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were determined using high-performance liquid chromatography with electrochemical detection [41].

### 2.10. Statistical analysis

All data were expressed as the means ± S.E.M. The analysis of body weight during the period of cross-fostering was conducted with a two-way analysis of variance (ANOVA), followed by Bonferroni's test as a *post hoc* comparison. Other statistical differences were tested using a one-way ANOVA followed by Bonferroni's test. A probability level of $P < 0.05$ was regarded as statistically significant.

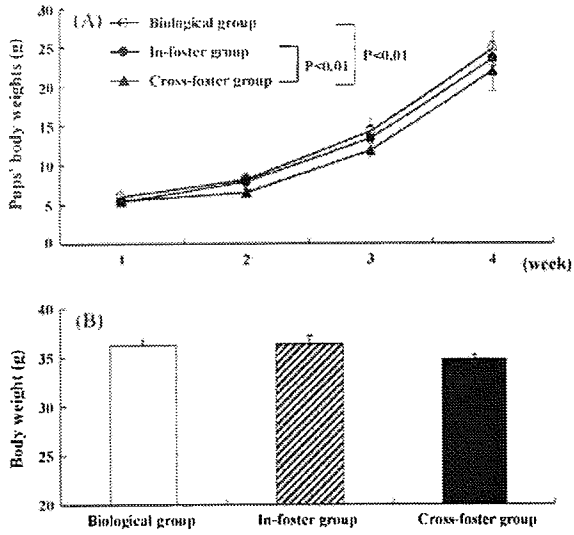


**Fig. 1.** Effect of cross-fostering on body weight. The body weight of pups during the cross-fostering period from 1 to 4 weeks (A) and at the age of 8 weeks (B). Data are expressed as the mean ± S.E.M. for 10–18 mice (Bonferroni's test).

## 3. Results

### 3.1. Effect of cross-fostering on body weight

As reported previously, body weight was considered as an assessment of cross-fostering, since it was sensitive to a change of rearing conditions [12]. To confirm the effect of cross-fostering, the weight of pups was measured during the period of cross-fostering and throughout their development. As shown in Fig. 1, the pups in the in-foster group gained weight as observed in the biological group. However, body weight was significantly lower in the cross-foster group than the biological or in-foster group during the period of cross-fostering ($F_{group(2,168)} = 22.40$, $P < 0.01$; $F_{week(3,168)} = 1394.66$, $P < 0.01$; $F_{group \times week(6,168)} = 2.13$, $P > 0.05$, Fig. 1A). No significant differences were observed among the three groups at the age of 8 weeks when all of the behavioral tests were started ($F_{(2,44)} = 1.80$, $P > 0.05$, Fig. 1B).

### 3.2. Effect of cross-fostering on behavior in the open-field test

An open-field test under mild stressful conditions is commonly used to detect emotional changes in mice [40]. In-fostered mice showed no changes of behavior in the open-field test compared with biological mice (Fig. 2). Meanwhile, the mice in the cross-foster group spent less time in the center squares ($F_{(2,43)} = 11.87$, $P < 0.01$, Fig. 2B) but longer in the corners ($F_{(2,43)} = 3.61$, $P < 0.05$, Fig. 2C) than either the biological or in-foster group when exposed to a novel environment under mild stressful conditions in the open-field. Furthermore, cross-fostered mice showed significant decreases in ambulation ($F_{(2,43)} = 5.26$, $P < 0.01$, Fig. 2D) and rearing ($F_{(2,43)} = 7.03$, $P < 0.01$, Fig. 2E) compared with the in-foster group. There were no significant differences in the latency to the center squares ($F_{(2,43)} = 0.37$, $P > 0.05$, Fig. 2A), the number of grooming events ($F_{(2,43)} = 0.55$, $P > 0.05$, Fig. 2F), and the frequency of defecation ($F_{(2,43)} = 0.12$, $P > 0.05$, Fig. 2G) as well as urination ($F_{(2,43)} = 0.72$, $P > 0.05$, Fig. 2H) in the cross-foster group (Fig. 2).

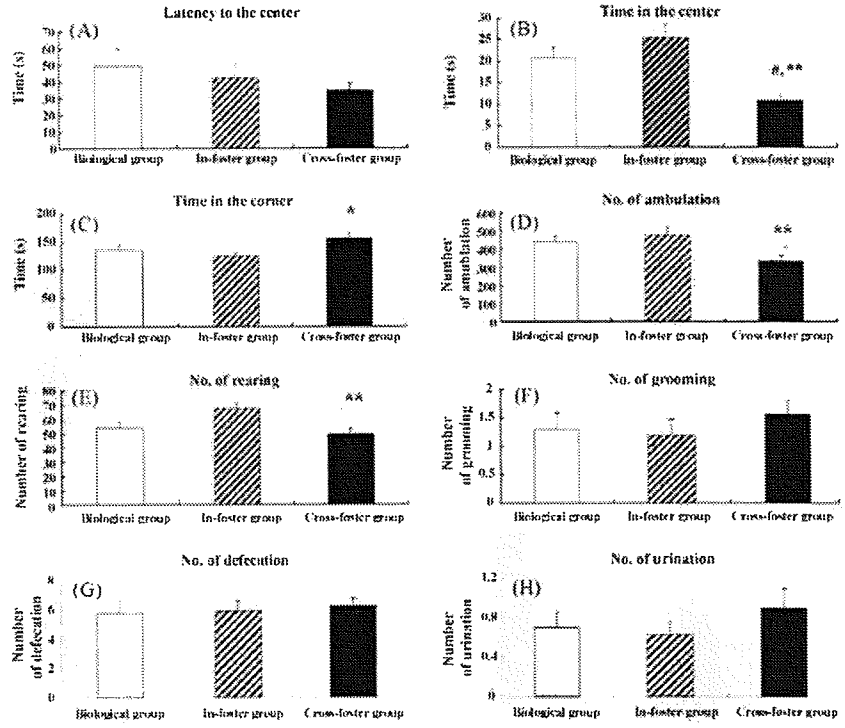


**Fig. 2.** Effect of cross-fostering on behavior in the open-field test. Time of latency to the center (A). Time spent in the center (B). Time spent in the corner (C). The number of times ambulation occurred (D). The number of times rearing occurred (E). The number of times grooming occurred (F). The number of times defecation occurred (G). The number of times urination occurred (H). Data are expressed as the mean ± S.E.M. for 10–18 mice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. in-foster group; $^{\#}P < 0.05$ vs. biological group (Bonferroni's test).

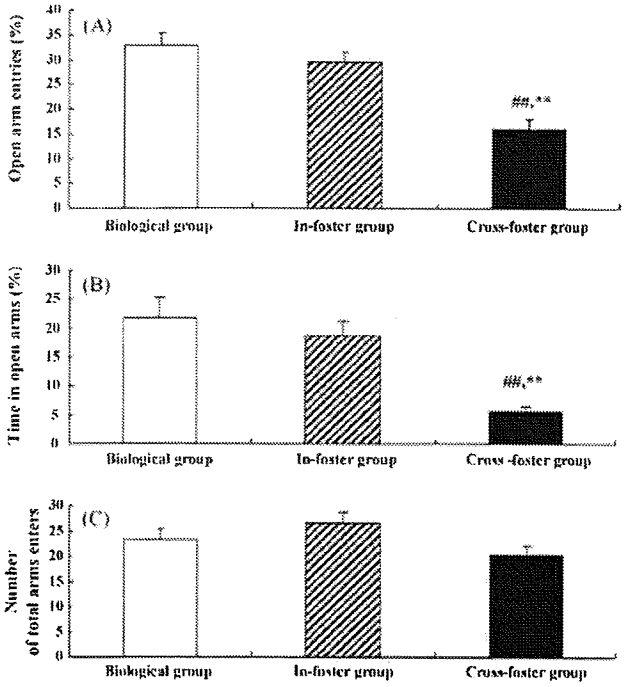


**Fig. 3.** Effect of cross-fostering on the behavior in the elevated plus maze test. Relative percentage of open arm entries (A). Relative percentage of time spent in open arms (B). Total number of arm entries (C). Data are expressed as the mean ± S.E.M. for 10–18 mice. $^{**}P<0.01$ vs. in-foster group mice; $^{\#\#}P<0.01$ vs. biological group (Bonferroni's test).

### 3.3. *Effect of cross-fostering on behavior in the elevated plus maze*

In the in-foster group, no significant changes in behavior in the elevated plus maze were observed compared with that in the biological group (Fig. 3). The percentage of open arm entries was significantly decreased in the cross-foster group compared with both the biological and the in-foster group ($F_{(2,44)}=15.90$, $P<0.01$, Fig. 3A). Notably, the percentage of time spent in the open arms was remarkably reduced in the cross-foster group compared with the other two control groups ($F_{(2,44)}=14.06$, $P<0.01$, Fig. 3B) whereas total arm entries was unaffected ($F_{(2,44)}=2.79$, $P>0.05$, Fig. 3C).

### 3.4. *Effect of cross-fostering on immobility time in the forced swimming test*

To further investigate the emotional response to stress, we examined the effect of cross-fostering on forced swimming-induced immobility. As shown in Fig. 4, the cross-fostered mice showed a significant increase of immobility time in the first 5 min

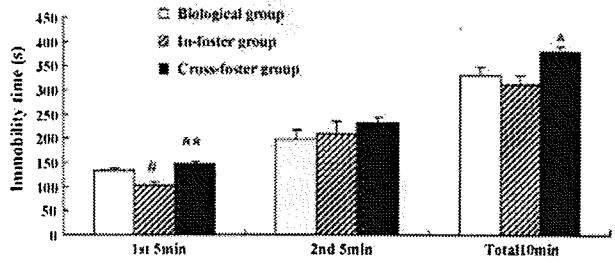


**Fig. 4.** Effect of cross-fostering on immobility time in the forced swimming test. Data are expressed as the mean ± S.E.M. for 10–17 mice. $^{*}P<0.05$, $^{**}P<0.01$ vs. in-foster group; $^{\#}P<0.05$ vs. biological group (Bonferroni's test).

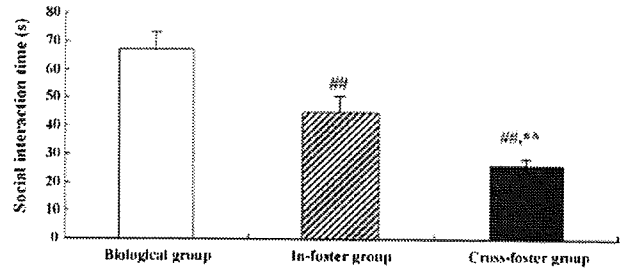


**Fig. 5.** Effect of cross-fostering on social interaction. Active social interaction behaviors, such as sniffing and grooming the partner, following, mounting, and crawling under or over the partner were recorded as the time of interaction during this period. Data are expressed as the mean ± S.E.M. for 10–17 mice. $^{**}P<0.01$ vs. in-foster group; $^{\#\#}P<0.01$ vs. biological group (Bonferroni's test).

and total 10 min but not the second 5 min (first 5 min: $F_{(2,42)}=9.06$, $P<0.01$; second 5 min: $F_{(2,42)}=0.74$, $P=0.48$, $P>0.05$; total 10 min: $F_{(2,43)}=4.88$, $P<0.05$, Fig. 4), compared with the in-fostered mice, which implied a state of increased depression which affected adaptation to stress. On the contrary, in-fostered mice showed a significant reduction of immobility time compared with biological control mice in the first 5 min, but not the second 5 min and total 10 min ($P<0.05$, Fig. 4).

### 3.5. *Effect of cross-fostering on social interaction*

The in-foster group showed a significant decrease in social interaction behavior compared with the biological group (Fig. 5). In addition, social interaction time was significantly shorter in the cross-foster group than the biological and in-foster groups ($F_{(2,42)}=17.84$, $P<0.01$, Fig. 5).

### 3.6. *Effect of cross-fostering on behavior in the Y-maze test*

There were no significant differences in spontaneous alternation behavior among the biological, in-fostered and cross-fostered mice in the Y-maze test (Fig. 6). The total number of arm entries was also unchanged among the three groups ($F_{(2,44)}=0.40$, $P>0.05$; $F_{(2,44)}=2.75$, $P>0.05$, respectively, Fig. 6).

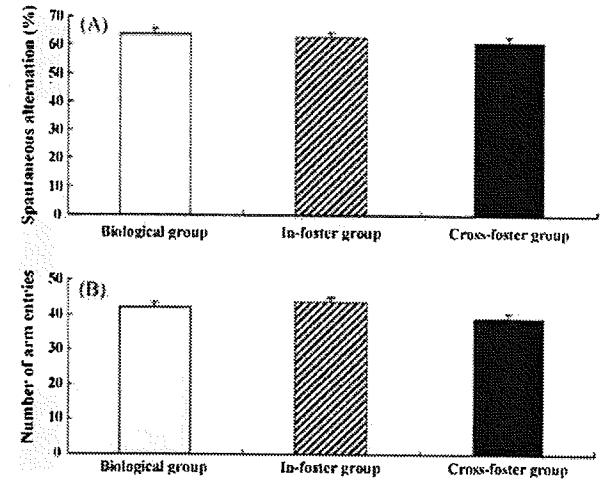


**Fig. 6.** Effect of cross-fostering on behavior in the Y-maze test. The percentage of spontaneous alternation behavior (A). The number of arm entries (B). Data are expressed as the mean ± S.E.M. for 10–18 mice. There were no significant differences among the groups (Bonferroni's test).

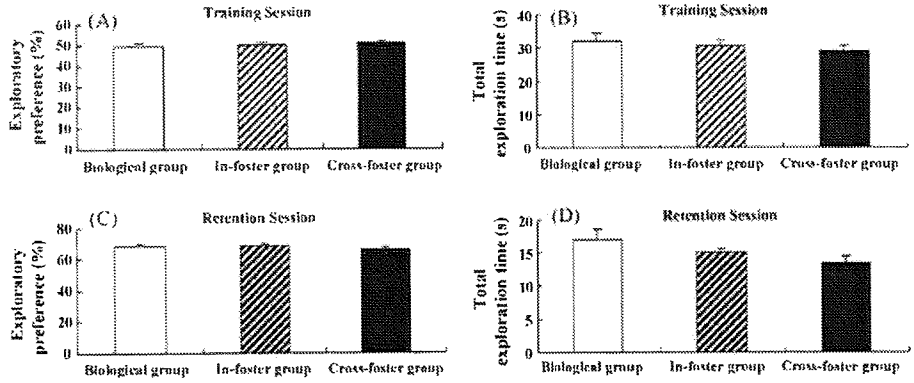


Fig. 7. Effect of cross-fostering on performance in the novel object recognition test. The percentage of exploratory preference in the training session (A). Total exploratory time in the training session (B). The percentage of exploratory preference in the retention session (C). Total exploratory time in the retention session (D). Data are expressed as the mean ± S.E.M. for 10–18 mice. There were no significant differences among the groups (Bonferroni's test).

### 3.7. Effect of cross-fostering on performance in the novel object recognition test

In the training session, the mice in the biological, in-foster and cross-foster groups spent equal amounts of time exploring either of the two objects ($F_{(2,44)} = 0.21$, $P > 0.05$, Fig. 7A), and thus there was no biased exploratory preference in either group of animals. In addition, total time spent in the exploration of objects in the training session did not differ among the three groups ($F_{(2,44)} = 0.55$, $P > 0.05$, Fig. 7B).

When retention performance was tested 24 h after the training session, three were no differences in the level of exploratory preference for the novel objects among the three groups ($F_{(2,44)} = 0.93$, $P > 0.05$, Fig. 7C). The total exploration time did not differ among the three groups in the retention session either ($F_{(2,44)} = 2.55$, $P > 0.05$, Fig. 7D).

### 3.8. Alteration of monoamine metabolism in the prefrontal cortex and hippocampus

To clarify the neurochemical basis of altered emotional behavior in cross-fostered mice, the amount of monoamines and their metabolites in the prefrontal cortex and hippocampus were determined. As shown in Fig. 8A, a significant decease of NE, 5-HT and its metabolites 5-HIAA as well as DA in the prefrontal cortex in cross-foster group was observed, compared with those in biological group (NE: $F_{(2,22)} = 3.93$, $P < 0.05$; 5-HT: $F_{(2,22)} = 3.80$, $P < 0.05$; 5-HIAA: $F_{(2,22)} = 6.07$, $P < 0.01$; DA: $F_{(2,22)} = 4.48$, $P < 0.05$, Fig. 8A). But, there were no differences for the contents of dopamine metabolites among groups, including DOPAC and HVA (DOPAC: $F_{(2,22)} = 1.16$, $P > 0.05$; HVA: $F_{(2,22)} = 0.94$, $P > 0.05$, Fig. 8A). In the hippocampus, compared with the biological group, the mice in cross-foster group also showed significant reduction of 5-HIAA ($F_{(2,25)} = 7.00$, $P < 0.01$, Fig. 8B), but no changes in NE, 5-HT, DA, DOPAC and HVA (NE: $F_{(2,25)} = 0.88$, $P > 0.05$; 5-HT: $F_{(2,25)} = 2.06$, $P > 0.05$; DA: $F_{(2,22)} = 2.92$, $P > 0.05$; DOPAC: $F_{(2,25)} = 0.63$, $P > 0.05$; HVA: $F_{(2,25)} = 1.87$, $P > 0.05$, Fig. 8B). For the in-foster group, the contents of NE in the prefrontal cortex and 5-HIAA in the hippocampus were also reduced compared with biological group (Fig. 8A and B). Whereas, the turnovers of monoaminergic neuronal systems were not affected by in- and cross-fosterings (data were not showed).

## 4. Discussion

Cross-fostering as a kind of early-life stress in rodents could mimic the psychology of children adopted as babies or suffering neglect as well as physical abuse [34]. Although most adopted individuals are well adjusted, population-based studies have reported an elevated risk for psychological maladjustment in adopted children compared with representative samples of nonadopted children [15]. A meta-analysis of findings from more than 25,000 adoptees, revealed significantly more behavioral and emotional problems among adoptees than nonadoptees [38]. Studies in animal models have found that cross-fostering within 24 h after birth affects the maternal behavior and pups' responses [11,30]. But, few articles have systemically examined its long-lasting effects on emotional or cognitive functions in ICR mice.

Clinical researches have reported that approximately 120,000 children in the USA are adopted annually, and adopted individuals constitute about 1.5 million children at young age [26]. However, the face of adoption is changing from decreasing domestic adoptions to a sharp increasing of international ones. Worldwide, approximately 40,000 children per year are moved between more than 100 countries through adoption [14]. Therefore, there is a persistent concern that adopted children may be at heightened risk for mental health or adjustment problems [16]. To clarify this concern, we designed the in- and cross-fostering groups to be equivalent to

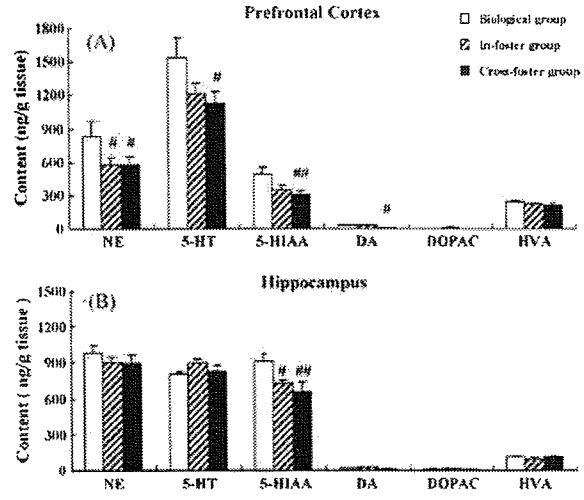


Fig. 8. Monoamines and their metabolite contents in the prefrontal cortex and hippocampus. The contents of monoamines and their metabolites in the prefrontal cortex (A). The monoamines and their metabolite contents in the hippocampus (B). Data are expressed as the mean ± S.E.M. for 8–10 mice. $^{**}P < 0.01$ vs. in-foster group; $^{*}P < 0.05$, $^{\#\#}P < 0.01$ vs. biological group (Bonferroni's test).

'the domestic' and 'international adoptions', respectively. Furthermore, we also think the degree of stress induced by adoption might be different for both pups and their adopted dams from in-fostering and biological groups. Therefore, it might produce different effects on long-term behavioral abnormality in each group. Some of our results showed these differences in behaviors and neurochemical parameters among them.

Previous studies have shown that adoption at different points in postnatal period affect the responses of pups and dams in rats: Early adoption on PD1 prevents the stress-induced secretion of corticosterone which is observed in offspring separated early, reduces locomotor activity in a novel environment, and improves spatial recognitive function [3,8]. In contract, later adoptions (PD5 and PD12) prolong the stress-induced secretion of corticosterone, increase locomotor response to novelty, and disrupt spatial recognition [3,8]. Furthermore, the second postnatal week has been reported as critical to establish the proper responses to later stress in adolescence [22]. Therefore, cross-fostering was carried out from PD7 to P28 in the present study to focus on the emotional behavior of offspring in adulthood.

Body weight during development has been considered as an indicator of maternal rearing [12]. In this study, we measured the body weight of pups throughout their development. No significant differences were observed at the age of 8 weeks when all of the behavioral tests were started. Therefore, it is unlikely that the behavioral changes in cross-fostered mice are due to malnutrition in adulthood. On the contrary, the body weight of ICR pups was reduced by cross-fostering during the period of adoption, indicating the maternal rearing by C57BL/6 dams is impaired by the adoption of a different strain of pups and the ICR pups is sensitive to adoption by C57BL/6 dams. Other possible explanations for the decrease in pups' body weight include the number of litters, the quality of adopted maternal care and the nourishment in milk, etc. [37], since we have used different strain of mice and alternated the relationship of dam and pups during their developing period. In this paper, we merely want to show the final co-effects of these factors in the first step of experiments. Therefore, we did not separate the exact role for each factor. Further researches are needed to clarify whether each factor differently influences on body weight in cross-fostering.

The changes in emotional behavior of mice were examined by the open-field, elevated plus maze and forced swimming as well as social interaction tests. In the open-field test, the mice are exposed to aversive stimuli (novelty, lit, and open area) to avoid and new places to explore. The internal conflict is measured by the duration of stay in peripheral areas and avoidance of the center area, called thigmotaxis, and the number of ambulation or rearing events is thought to reflect the exploratory tendencies in mice [35]. In the present study, the cross-fostered mice showed an increase in thigmotaxis and decrease in exploratory tendencies. The results were consistent with the previous report that C57BL/6By] mice raised by BALB/cBy] dams spent less time in the center of the open-field area [30]. Furthermore, in the elevated plus maze test, the cross-fostered mice showed a decrease in the both of the number of open arm entries and the time spent in open arms, parameters of potential anxiety in mice [18]. Taken together, these behavioral studies suggested the cross-fostered mice are in a state of increased anxiety. Immobility time in forced swimming test is used to evaluate the state of stress or depression since it can be increased by stressors and reversed by some antidepressants [9]. The cross-fostered mice showed a significant enhancement of immobility time during the first 5 min in the forced swimming test which suggests increased sensitivity to stress or inappropriate coping responses when facing severely stressful situations. Social withdrawal is regarded as a feature of emotional disorders [19]. We examined the social behavior of offspring in adulthood. The social interaction time in the cross-foster group was remarkably shorter than that in the in-foster group. Interestingly, the mice not only in cross-foster group but also in-foster group showed a significant decrease in social interaction behavior compared with those in the biological control group, suggesting the social interaction test may be more sensitive for detecting the behavioral changes of fostering. Taken together, all of the behavioral results indicate that the cross-fostered mice were in a state of high emotionality and so failed to adapt to other stressors.

Cognitive function was also evaluated by measuring the spontaneous alternation behavior of mice in the Y-maze test (an index of spatial memory) and exploratory preference in the novel object recognition test (an index of visual recognition memory) [25]. There were no significant differences in cognitive function among the different fostering groups suggesting that the cross-fostered mice were normal in cognitive function including short-term memory and visual recognition memory in ICR mice. However, the data supporting unaffected memory are inconclusive: later adoption from PD5 or PD12 has been reported to impair memory in the Y-maze test for male adult offspring in rats, whereas early adoption from PD1 has the opposite effect [3]. The differences may be partly due to either the period of cross-fostering or the strains of mice in each study. Therefore, we cannot make such conclusion that the stress of cross-fostering might not be strong enough to impair learning or memory in ICR mice. Other types of memory should be tested such as spatial memory, contextual memory, latent learning associated with selective attention and motor learning.

In the present study, we used the same offspring for behavioral tests, repeatedly, to measure various emotional and cognitive functions. It is unlikely that the carry-over effects of previous behavioral tests would affect the following behavioral tests, since (1) each behavioral test consists of different parameters which affect motivation and curiosity, etc., (2) all groups have the same influences from previous behavioral tests and (3) we compared them with the control one. The control group in the present study did not show any behavioral abnormality compared with that in our previous studies [23,25,40].

To investigate the neurochemical basis of these emotional abnormalities, we measured the levels of monoamine neurotransmitters and their metabolites in the prefrontal cortex and hippocampus, which related to emotional and cognitive function [40]. We found the contents of 5-HT and 5-HIAA were reduced in cross-fostered mice, indicating the deficit of serotoninergic neuronal function by cross-fostering. It is well known the serotonergic system plays a critical role in regulation of emotional stress during development, and the dysfunction of serotonergic system has been implicated in the etiology of emotional disorders [16,22]. Therefore, the aberrant serotonergic system or its receptors induced by cross-fostering may lead to these emotional abnormalities. Furthermore, since the dysfunctions of noradrenergic and dopaminergic system are also related to some stress-induced disorders, such as depression [5,24], the reduction of NE and DA in the prefrontal cortex might partly contribute to these emotional abnormalities of cross-fostered mice in the present study. However, further research is needed to determine the precise mechanisms of cross-fostering on impaired emotional behavior in ICR mice.

## 5. Conclusion

In conclusion, the present study demonstrated that cross-fostering of ICR pups with C57BL/6 dams from PD7 for 3 weeks affected the emotionality, but not memory, of offspring in adulthood which could mimic the psychology of adoption in humans. Furthermore, stress-related psychological diseases are known to

involve both genetic susceptibility and environmental factors, but the interactions of genes and the environment in the susceptibility to stress are still unclear [7]. The early-life stress induced by cross-fostering has important implications for research into vulnerability to stress or the interactions of genetic and environmental factors using stress or psychiatric disease-related genetic animal models.

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## References

[1] Abe H, Hidaka N, Kawagoe C, Odagiri K, Watanabe Y, Ikeda T. Prenatal psychological stress causes higher emotionality, depression-like behavior, and elevated activity in the hypothalamo-pituitary-adrenal axis. Neurosci Res 2007;59:145–51.
[2] Anisman H, Zaharia MD, Meaney MJ, Merali Z. Do early-life events permanently alter behavioral and hormonal responses to stressors? Int J Dev Neurosci 1998;16:149–64.
[3] Barbazanges A, Vallée M, Mayo W, Day J, Simon H, Le Moal M, et al. Early and later adoptions have different long-term effects on male rat offspring. J Neurosci 1996;16:7783–90.
[4] Beckett C, Maughan B, Rutter M, Castle J, Colvert E, Groothues C, et al. Do the effects of early severe deprivation on cognition persist into early adolescence? Findings from the English and Romanian adoptees study. Child Dev 2006;77(3):696–711.
[5] Birnbaum S, Gobeske KT, Auerbach J, Taylor JR, Arnsten AF. A role for norepinephrine in stress-induced cognitive deficits: α-1-adrenoceptor mediation in the prefrontal cortex. Biol Psychiatry 1999;46:1266–74.
[6] Cannon TD, van Erp TG, Bearden CE, Loewy R, Thompson P, Toga AW, et al. Early and late neurodevelopmental influences in the prodrome to schizophrenia: contributions of genes, environment, and their interactions. Schizophr Bull 2003;29(4):653–69.
[7] Charney DS. Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. Am J Psychiatry 2004;161:195–216.
[8] Damaudéry M, Koehl M, Barbazanges A, Cabib S, Le Moal M, Maccari S. Early and later adoptions differently modify mother–pup interactions. Behav Neurosci 2004;118:590–6.
[9] Enthoven L, de Kloet ER, Oitzl MS. Effects of maternal deprivation of CD1 mice on performance in the water maze and swim stress. Behav Brain Res 2008;187:195–9.
[10] Francis DD, Szegda K, Campbell G, Martin WD, Insel TR. Epigenetic sources of behavioral differences in mice. Nat Neurosci 2003;6(5):445–6.
[11] Friedman E, Berman M, Overstreet D. Swim test immobility in a genetic rat model of depression in modified by maternal environment: a cross-foster study. Dev Psychobiol 2006;48:169–77.
[12] Gomez-Serrano M, Tonelli L, Listwak S, Sternberg E, Riley AL. Effects of cross fostering on open-field behavior, acoustic startle, lipopolysaccharide-induced corticosterone release, and body weight in Lewis and Fischer rats. Behav Genet 2001;31:427–36.
[13] Heim C, Nemeroff CB. The impact of early adverse experiences on brain systems involved in the pathophysiology of anxiety and affective disorders. Biol Psychiatry 1999;46:1509–22.
[14] Juffer F, van Ijzendoorn MH. Behavior problems and mental health referrals of international adoptees: a meta-analysis. JAMA 2005;293:2501–15.
[15] Keyes MA, Sharma A, Elkins IJ, Iacono WG, McGue M. The mental health of US adolescents adopted in infancy. Arch Pediatr Adolesc Med 2008;162(5):419–25.
[16] Konno K, Matsumoto M, Togashi H, Yamaguchi T, Izumi T, Watanabe M, et al. Early postnatal stress affects the serotonergic function in the median raphe nuclei of adult rats. Brain Res 2007;1172:60–6.
[17] Lehmann J, Pryce CR, Jongen-Relo AL, Stohr T, Pothuizen HH, Feldon J. Comparison of maternal separation and early handling in terms of their neurobehavioral effects in aged rats. Neurobiol Aging 2002;23:457–66.
[18] Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 1987;92:180–5.
[19] Lysaker PH, Salyers MP. Anxiety symptoms in schizophrenia spectrum disorders: associations with social function, positive and negative symptoms, hope and trauma history. Acta Psychiatr Scand 2007;116(4):290–8.
[20] Maccari S, Morley-Fletcher S. Effects of prenatal restraint stress on the hypothalamus–pituitary–adrenal axis and related behavioural and neurobiological alterations. Psychoneuroendocrinology 2007;32(Suppl. 1):S10–5.
[21] Mamiya T, Kise M, Morikawa K. Ferulic acid attenuated cognitive deficits and increase in carbonyl proteins induced by buthionine-sulfoximine in mice. Neurosci Lett 2008;430:115–8.
[22] Matsumoto M, Higuchi K, Togashi H, Koseki H, Yamaguchi T, Kanno M. Early postnatal stress alters the 5-HTergic modulation to emotional stress at postadolescent periods of rats. Hippocampus 2005;15:775–81.
[23] Miyagawa H, Hasegawa M, Fukuta T, Amano M, Yamada K, Nabeshima T. Dissociation of impairment between spatial memory, and motor function and emotional behavior in aged rats. Behav Brain Res 1998;91:73–81.
[24] Mizoguchi K, Shoji H, Ikeda R, Tanaka Y, Tabira T. Persistent depressive state after chronic stress in rats is accompanied by HPA axis dysregulation and reduced prefrontal dopaminergic neurotransmission. Pharmacol Biochem Behav 2008;91(1):170–5.
[25] Mouri A, Noda Y, Hara H, Mizoguchi H, Tabira T, Nabeshima T. Oral vaccination with a viral vector containing Abeta cDNA attenuates age-related Abeta accumulation and memory deficits without causing inflammation in a mouse Alzheimer model. FASEB J 2007;21:2135–48.
[26] Nickman SL, Rosenfeld AA, Fine P, Macintyre JC, Pilowsky DJ, Howe RA, et al. Children in adoptive families: overview and update. J Am Acad Child Adolesc Psychiatry 2005;44(10):987–95.
[27] Noda Y, Yamada K, Furukawa H, Nabeshima T. Enhancement of immobility in a forced swimming test by subacute or repeated treatment with phencyclidine: a new model of schizophrenia. Br J Pharmacol 1995;116:2531–7.
[28] Nulman I, Rovet J, Greenbaum R, Loebstein M, Wolpin J, Pace-Asciak P, et al. Neurodevelopment of adopted children exposed in utero to cocaine: the Toronto Adoption Study. Clin Invest Med 2001;24(3):129–37.
[29] Pascual R, Zamora-León SP. Effects of neonatal maternal deprivation and postweaning environmental complexity on dendritic morphology of prefrontal pyramidal neurons in the rat. Acta Neurobiol Exp (Wars) 2007;67(4):471–9.
[30] Priebe K, Brake WG, Romeo RD, Sisti HM, Mueller A, McEwen BS, et al. Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/CJ mice: a cross-fostering study. Dev Psychobiol 2005;47:398–407.
[31] Qiao H, Noda Y, Kamei H, Nagai T, Furukawa H, Miura H, et al. Clozapine, but not haloperidol, reverses social behavior deficit in mice during withdrawal from chronic phencyclidine treatment. Neuroreport 2001;12:11–5.
[32] Sharma AR, McGue M, Benson P. The emotional and behavioral adjustment of United States adopted adolescents, part II: age at placement. Child Youth Serv Rev 1996;18(1–2):101–14.
[33] Son GH, Geum D, Chung S, Kim EJ, Jo JH, Kim CM, et al. Maternal stress produces learning deficits associated with impairment of NMDA receptor-mediated synaptic plasticity. J Neurosci 2006;22–26:3309–18.
[34] Sullivan R, Wilson DA, Feldon J, Yee BK, Meyer U, Richter-Levin G, et al. The International Society for Developmental Psychobiology Annual Meeting Symposium: impact of early life experiences on brain and behavioral development. Dev Psychobiol 2006;48:583–602.
[35] Treit D, Fundytus M. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacol Biochem Behav 1988;31:959–62.
[36] Vallée M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. J Neurosci 1997;17:2626–36.
[37] Van der Veen R, Abrous DN, de Kloet ER, Piazza PV, Koehl M. Impact of intra- and interstrain cross-fostering on mouse maternal care. Genes Brain Behav 2008;7:184–92.
[38] Wierzbicki M. Psychological adjustment of adoptees: a meta-analysis. J Clin Child Psychol 1993;22(4):447–54.
[39] Wigger A, Loerscher P, Weissenbacher P, Holsboer F, Landgraf R. Cross-fostering and cross-breeding of HAB and LAB rats: a genetic rat model of anxiety. Behav Genet 2001;31:371–82.
[40] Yamada K, Iida R, Miyamoto Y, Saito K, Sekikawa K, Seishima M, et al. Neurobehavioral alterations in mice with a targeted deletion of the tumor necrosis factor-alpha gene: implications for emotional behavior. J Neuroimmunol 2000;111:131–8.
[41] Yamada K, Noda Y, Nakayama S, Komori Y, Sugihara H, Hasegawa H, et al. Role of nitric oxide in learning and memory and in monoamine metabolism in the rat brain. Br J Pharmacol 1995;115:852–8.

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Research report

# Behavioral abnormality and pharmacologic response in social isolation-reared mice

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Social interaction

ABSTRACT

Social isolation (SI) rearing in rodents causes a variety of behavioral changes, including hyperlocomotion, anxiety, impulsivity, aggression, and learning and memory deficits. These behavioral abnormalities in rodents may be related to the symptoms in patients with neuropsychiatric disorders, such as attention-deficit hyperactivity disorder, obsessive-compulsive disorder, autism, schizophrenia and depression. In this study, we examined the effect of long-term SI rearing after weaning on emotional behaviors and cognitive function in mice. Furthermore, the effects of methylphenidate (MPH), clozapine (CLZ) and fluoxetine (FLX) on SI-induced behavioral changes were examined to measure the predictive validity of SI-reared mice as an animal model for these neuropsychiatric disorders. MPH improved SI-induced anxiety-like behavior in the elevated-plus maze test, but had no effect on aggressive behavior. In contrast, CLZ ameliorated aggressive behavior, but not anxiety-like behavior in SI-reared mice. Repeated FLX treatment prevented SI-induced aggressive behavior and social interaction deficits. These findings suggest that SI-induced behavioral abnormality is a psychobehavioral complex relevant to various clinical symptoms observed in neuropsychiatric disorders and that SI-reared mice are a useful animal model to study the pathophysiology/pathogenesis of these diseases.



## 1. Introduction

Adverse early life experiences, such as maternal separation or social isolation (SI) affect structural and functional brain development and adult behaviors in rodents [22,31,38]. Behavioral changes induced by SI rearing have been characterized, including enhanced locomotor activity under a novel environment [47,49], anxiety-like behavior [23,48], aggressive behavior [24,29,50], and impairment of prepulse inhibition of the acoustic startle response [9] and spatial learning and memory in the Morris water maze [24,28].

The social environment in early life significantly influences not only the behavioral organization but also neurochemical and anatomical development of the brain. For instance, dopamine and serotonin systems are affected by SI in the nucleus accumbens [20], prefrontal cortex [21] and hippocampus [33]. The neuroanatomical consequences of isolation rearing include decreased spine density of pyramidal neurons in the prefrontal cortex and hippocampus [41], fewer hippocampal synapses [46] and the decreased survival of newly divided cells and neurogenesis in the dentate gyrus of hippocampus [24].

The behavioral, neurochemical and anatomical changes in SI-reared mice may be related to clinical symptoms and pathophysiology in patients with neuropsychiatric disorders [15] in which anxiety, impulsivity and aggression are commonly observed. To address this issue, we measured the predictive validity in SI-reared mice by examining the effects of methylphenidate (MPH), clozapine (CLZ) and fluoxetine (FLX) on SI-induced behavioral abnormality. A clinical report has shown that attention-deficit/hyperactivity disorder (ADHD) occurred with disruptive disorders (oppositional defiant disorder or conduct disorder), internalizing disorder (anxiety and/or depression), or both [25]. MPH is one of the most commonly used drugs to treat ADHD [4] and is effective to improve attention and behavior, including impulsivity and aggression [34,43]. CLZ, atypical antipsychotic, is effective to reduce

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aggressive or violent acts in schizophrenia and neuroleptic-resistant schizophrenia patients [17,42]. FLX, a selective serotonin reuptake inhibitor (SSRI), is used to treat depression. In addition, FLX reduces impulsive aggressive behavior in personality-disordered subjects [7]. Our findings suggest that SI-induced behavioral abnormality is a psychobehavioral complex relevant to various clinical symptoms observed in neuropsychiatric disorders, including ADHD, schizophrenia and depression, and that SI-reared mice are a useful animal model to study the pathophysiology/pathogenesis of these diseases.

## 2. Materials and methods

### 2.1. Animals

Male ICR mice 3 and 7 weeks old (Japan SLC Inc., Hamamatsu, Japan) were purchased and used for the experiments. They were housed under a standard 12-h light/dark cycle (lights on 9:00 am) at a constant temperature of 23 ± 1 °C with free access to food and water throughout the experiments. The animals were handled in accordance with the guidelines established by the Institutional Animal Care and Use Committee of Nagoya University, the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### 2.2. Isolation rearing

After 3 days of acclimatization, 3-week-old mice were randomly divided into two groups: SI rearing and group-housed (GH) rearing. Mice in the SI group were individually housed in wire-topped opaque polypropylene cages (20 cm × 12 cm × 10 cm) while mice in the GH group continued to be housed under normal conditions (five per cage) in wire-topped clear plastic cages (34 cm × 22 cm × 15 cm). After 4 weeks SI, mice were subjected to behavioral analyses, as described below. During behavioral analysis, the housing conditions were maintained.

### 2.3. Drug administration

MPH hydrochloride (Nihon Ciba-Geigy K.K., Tokyo, Japan) and FLX (Sigma–Aldrich Co., St. Louis, MO, USA) were dissolved in saline. CLZ (Sigma–Aldrich Co.) was dissolved in a single drop of 1 N chloridric acid (HCl), diluted with saline, and neutralized by sodium bicarbonate [16]. MPH (1 and 3 mg/kg) and CLZ (0.5 and 2.5 mg/kg) were administered intraperitoneally (i.p.) 30 min before behavioral studies. Daily administration of FLX (10 mg/kg, i.p.) was started 2 weeks after SI, and continued until the end of the behavioral tests. During behavioral analysis, FLX was administered 30 min before the behavioral test [24].

### 2.4. Spontaneous locomotor activity under a novel environment

Locomotor activity was measured for 1 h using digital counters with an infrared detector (NS-AS01; Brain Science Idea Co., Ltd., Tokyo, Japan) in a polycarbonate box (35 cm × 30 cm × 17 cm high).

### 2.5. Elevated-plus maze test

The elevated-plus maze consisted of two open (25 cm × 8 cm × 0.5 cm) and two closed (25 cm × 8 cm × 20 cm) arms emanating from a common central platform (8 cm × 8 cm) to form a plus shape [32]. The entire apparatus was elevated to 50 cm above floor level and illuminated with a 20-W bulb. The test was started by placing a mouse on the central platform of the maze facing an open arm. An arm entry was defined as all four paws in the arm. The duration of time spent in any arms and number of arm entries was measured for 5 min. These data were used to calculate the percentage of duration in open arms [i.e., (duration in open arms/duration in open and closed arms) × 100].

### 2.6. Forced swim test

The forced swim test was carried out as described previously with minor modifications [27]. Mice were placed in a glass cylinder (20 cm high × 13.5 cm diameter) filled to a depth of 12 cm with water (24 ± 1 °C). A 3-min test per day was repeated for 5 days. Immobility time (floating) was measured. A mouse was judged to be

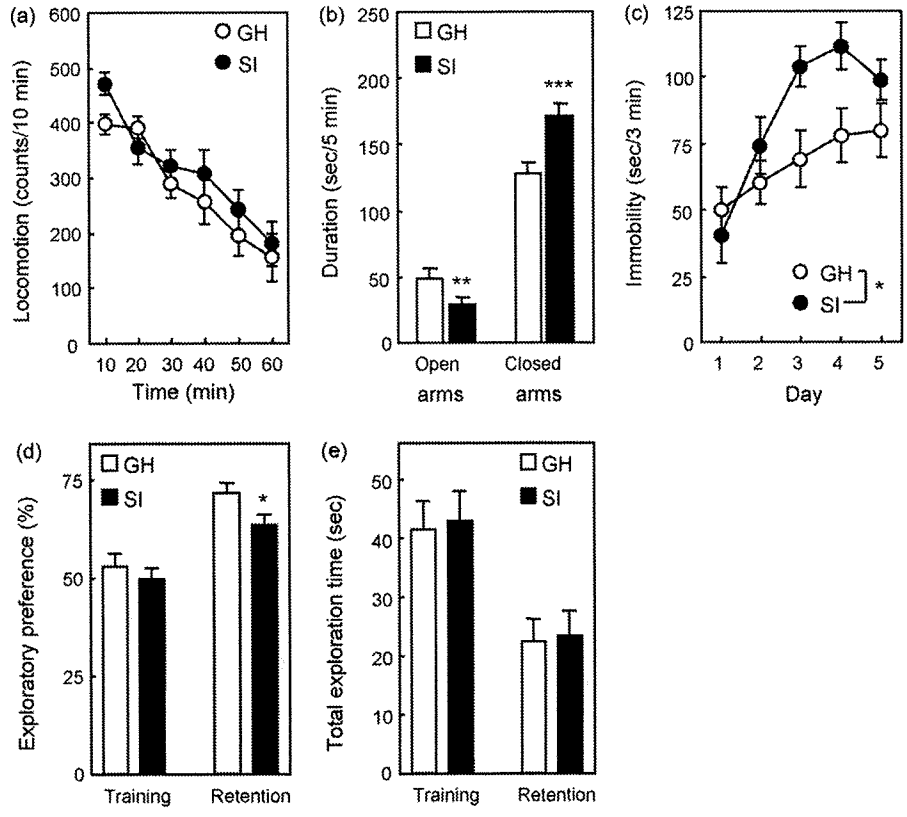


**Fig. 1.** Effect of social isolation (SI) rearing on emotional behavior and recognition memory. (a) Spontaneous locomotor activity under a novel environment. Locomotor activity was measured every 10 min for 60 min [group-housed (GH): $n = 10$, SI: $n = 11$]. Effect of rearing condition: $F(1,19) = 1.081$, $p = 0.3116$; effect of time (within-subject effect): $F(11,209) = 15.5$, $p < 0.001$; interaction: $F(11,209) = 1.071$, $p < 0.3861$. (b) Exploratory activity in the elevated-plus maze test. Exploratory activity was measured for 5 min (GH: $n = 17$, SI: $n = 19$). (c) Immobility in the forced swim test. Immobility was measured for 3 min per day (GH: $n = 9$, SI: $n = 10$). Effect of rearing condition: $F(1,17) = 5.228$, $p = 0.0354$; effect of day (within-subject effect): $F(4,68) = 11.821$, $p < 0.0001$; interaction: $F(4,68) = 2.316$, $p = 0.066$]. (d) Exploratory preference and (e) total exploration time in the novel object recognition test. Retention session was carried out 24 h after training (GH: $n = 14$, SI: $n = 15$). Data are shown as the means ± SE. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. GH mice [Student's $t$-test (b), (d) or ANOVA (c)].

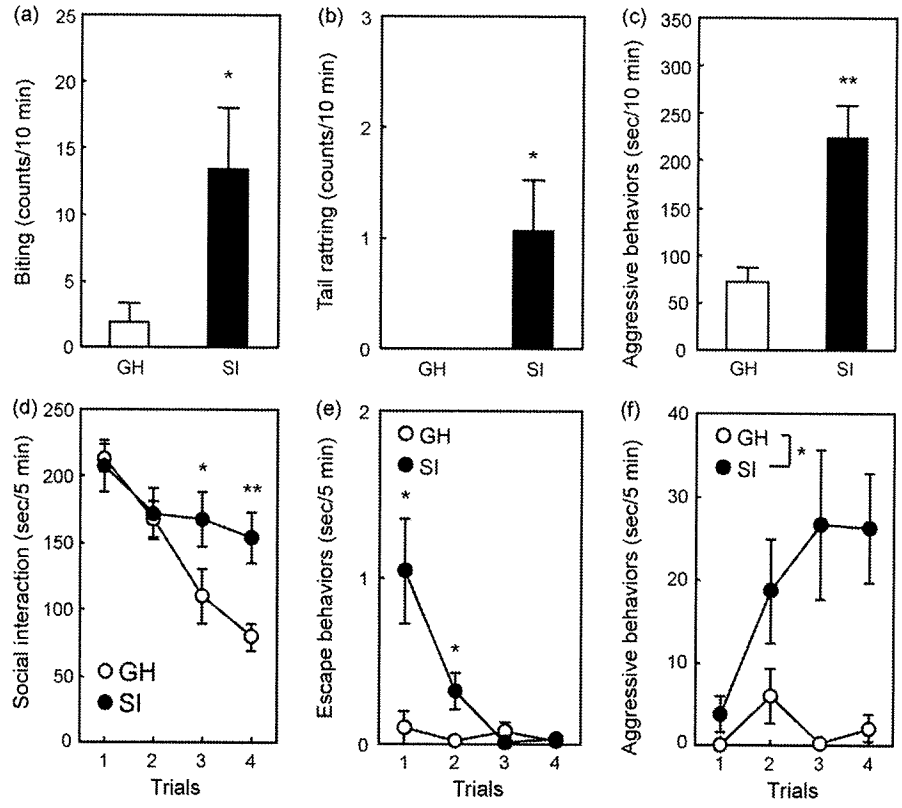


**Fig. 2.** Effect of social isolation (SI) rearing on aggressive and social behavior. (a–c) Aggressive behavior in the intruder-evoked aggressive test was measured for 10 min [group-housed (GH): $n = 15$, SI: $n = 15$]. (d–f) Social, escape and aggressive behavior was measured in four trials (5 min for each trial) with an intertrial interval of 30 min in the social interaction test (GH: $n = 13$, SI: $n = 15$). (d) Social interaction. Effect of rearing condition: $F(1,26) = 2.877$, $p = 0.1018$; effect of trial (within-subject effect): $F(3,78) = 25.446$, $p < 0.0001$; interaction: $F(3,111) = 6.261$, $p = 0.0007$. (e) Escape behavior. Effect of rearing condition: $F(1,26) = 8.877$, $p = 0.0062$; effect of trial (within-subject effect): $F(3,78) = 7.579$, $p = 0.0002$; interaction: $F(3.78) = 6.157$, $p = 0.0008$. (f) Aggressive behavior. Effect of rearing condition: $F(1,26) = 8.066$, $p = 0.0086$; effect of trial (within-subject effect): $F(3,78) = 4.013$, $p = 0.0104$; interaction: $F(3,78) = 1.601$, $p = 0.1959$. Data are shown as the means ± SE. $^*p < 0.05$, $^{**}p < 0.01$ vs. GH mice [Student's $t$-test (a–e) or ANOVA (f)].

immobile if it ceased struggling and remained floating motionless in water making only those movements necessary to keep its head above water.

### 2.7. Intruder-evoked aggressive test

The intruder-evoked aggressive test was carried out according to our previous report [24]. Male 7-week-old ICR mice were used as intruders which had not shown aggressive behavior against their peers. The resident mouse was habituated to the test cage (20 cm × 12 cm × 10 cm high) for 10 min, and then an intruder mouse was placed in the test cage. The investigating behavior of the resident mouse against the intruder was observed for 10 min. The frequency of attacking/biting and tail rattling, and duration of aggression, including attacking/biting, tail rattling, aggressive grooming, sideways posturing and pushing under were analyzed.

### 2.8. Social interaction test

To investigate the habituation response to a novel mouse, the social interaction test was carried out as described previously with minor modifications [44]. A male resident mouse was housed alone in a home cage (34 cm × 22 cm × 15 cm high) for 2 days before the test, and then a novel male mouse was introduced into the cage for 5 min per trial. The test consisted of four trials, in which test mice were exposed to the same novel mouse, with an intertrial interval of 30 min. We measured the time spent in social interaction (close following, inspection, anogenital sniffing, and other social body contact), escape behavior (actively avoiding the other mouse), and aggressive behavior (biting, wrestling and tail rattling).

### 2.9. Novel object recognition test

The novel object recognition test was carried out as described previously [35]. The experimental apparatus consisted of a Plexiglas open-field box (30 cm × 30 cm × 35 cm high), the floor of which was covered in sawdust. The apparatus was located in a sound-attenuated room and illuminated with a 20-W bulb. The test procedure consisted of three different sessions: habituation, training, and retention. Each mouse was individually habituated to the box, with 10 min exploration in the absence of objects for 3 consecutive days (habituation session, days 1–3). During the training session, two different novel objects were symmetrically fixed to the floor of the box, 8 cm from the walls, and each animal was allowed to explore in the box for 10 min (day 4). The objects were constructed from a golf ball, wooden column, and wall socket, which were different in shape and color but similar in size. The animals were considered to be exploring the object when the head of the animal was facing the object or when the animal was touching or sniffing the object. The time spent exploring each object was recorded. After training, mice were immediately returned to their home cages. During the retention sessions, the animals were placed back in the same box 24 h (day 5) after the training session, in which one of the familiar objects used during training was replaced by a novel object. The animals were then allowed to explore freely for 5 min, and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counterbalanced manner in terms of their physical complexity and emotional neutrality. A preference index, the ratio of the amount of time spent exploring any one of the two objects (training session) or the novel object (retention session) over the total time spent exploring both objects, was used to measure cognitive function.

### 2.10. Statistical analysis

Statistical analyses were performed using StatView 5.0 software (SAS Institute, Cary, NC, USA). For locomotor activity, the forced swim test and social interaction test, one-way repeated measure ANOVA was used. To analyze the drug's effect, two-way or two-way repeated measures ANOVA was performed. The rearing condition, drug, and dose were between-subject factors. Time (day or trial) was a within-subject factor. Significant main effects or interactions were followed by Bonferroni's *post hoc* test. Differences between two groups were analyzed by the two-tailed Student's $t$-test.

## 3. Results

### 3.1. SI rearing induced behavioral abnormality

There was no significant difference in spontaneous locomotor activity between GH and SI mice under novel environmental con-

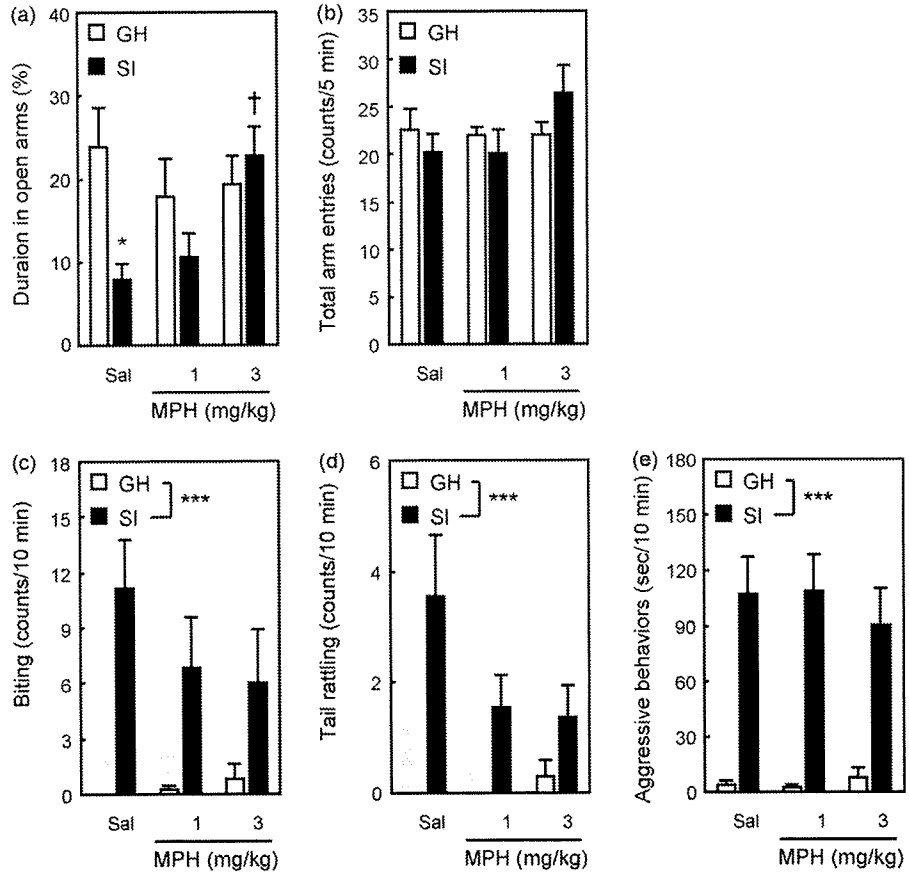


**Fig. 3.** Effect of methylphenidate (MPH) on social isolation (SI)-induced anxiety-like and aggressive behavior. MPH (1 or 3 mg/kg, i.p.) was administered 30 min before the behavioral test. (a–b) The elevated-plus maze test [saline-treated group-housed (GH) mice ($n = 10$); MPH (1 mg/kg)-treated GH mice ($n = 9$); MPH (3 mg/kg)-treated GH mice ($n = 10$); saline-treated SI mice ($n = 12$); MPH (1 mg/kg)-treated SI mice ($n = 10$); MPH (3 mg/kg)-treated SI mice ($n = 11$)]. (a) Duration in open arms. Effect of rearing condition: $F(1,56) = 5.238$, $p = 0.259$; effect of dose: $F(2,56) = 1.943$, $p = 0.1527$; interaction: $F(2,56) = 3.977$, $p = 0.0243$. (b) Total arm entries. Effect of rearing condition: $F(1,56) = 0.002$, $p = 0.9661$; effect of dose: $F(2,56) = 1.387$, $p = 0.2582$; interaction: $F(2,56) = 1.638$, $p = 0.2035$. (c)-(e) The intruder-evoked aggressive test [saline-treated GH mice ($n = 10$); MPH (1 mg/kg)-treated GH mice ($n = 9$); MPH (3 mg/kg)-treated GH mice ($n = 10$); saline-treated SI mice ($n = 11$); MPH (1 mg/kg)-treated SI mice ($n = 11$); MPH (3 mg/kg)-treated SI mice ($n = 11$)]. (c) Frequency of biting. Effect of rearing condition: $F(1,56) = 19.565$, $p < 0.0001$; effect of dose: $F(1,56) = 0.682$, $p = 0.5096$; interaction: $F(1,56) = 0.682$, $p = 0.5096$. (d) Frequency of tail rattling. Effect of rearing condition: $F(1,56) = 16.658$, $p = 0.0001$; effect of dose: $F(1,56) = 1.675$, $p = 0.1966$; interaction: $F(1,56) = 2.320$, $p = 0.1077$. (e) Duration of aggressive behaviors. Effect of rearing condition: $F(1,56) = 60.983$, $p < 0.0001$; effect of dose: $F(1,56) = 0.124$, $p = 0.8833$; interaction: $F(1,56) = 0.359$, $p = 0.6999$. Data are shown as the means ± SE. $^{*}p < 0.05$, $^{***}p < 0.001$ vs. GH mice [Bonferroni's *post hoc* test (a) or ANOVA (c–e)]. $^{\dagger}p < 0.05$ vs. saline-treated mice (Bonferroni's *post hoc* test).

ditions (Fig. 1a). In the elevated-plus maze test, the time spent in the open and closed arms was significantly different between GH and SI mice. SI mice spent significantly less time exploring the open arms and longer time in the closed arms than GH mice (Fig. 1b). In the forced swim test, one-way repeated ANOVA revealed a significant effect of the rearing condition on the immobility time, which was significantly increased in SI mice compared with GH mice (Fig. 1c).

In the novel object recognition test, SI rearing significantly reduced the exploratory preference to a novel object in the retention test (Fig. 1d). There was no difference in the total exploratory time in the training or retention sessions between the two groups (Fig. 1e).

In the intruder-evoked aggressive test, SI rearing significantly increased biting (Fig. 2a), tail rattling (Fig. 2b) and total time of aggressive behavior as compared with GH mice (Fig. 2c). Furthermore, GH mice showed a characteristic reduction of the time for investigating an intruder mouse. In contrast, SI mice showed little reduction in social interaction, suggesting impaired habituation to an unfamiliar intruder (Fig. 2d). SI rearing significantly increased escape behavior compared with GH mice in the first and second trials (Fig. 2e). Consistent with the result of the intruder-evoked aggressive test (Fig. 2a and b), SI mice exhibited significantly more aggressive behavior than GH mice (Fig. 2f).

### 3.2. MPH improved SI-induced anxiety-like behavior, but not aggressive behavior

MPH significantly ameliorated the reduced time spent in the open arms in SI mice in a dose-dependent manner (Fig. 3a) without affecting the total number of arm entries (Fig. 3b). MPH had no effect on SI-induced aggressive behavior at the dose examined (Fig. 3c), although there was a tendency for the number of biting and tail rattling in SI mice to decrease (Fig. 3a and b).

### 3.3. CLZ improved SI-induced aggressive behavior, but not anxiety-like behavior

CLZ (2.5 mg/kg) significantly decreased the time spent in the open arms and total arm entries in the elevated-plus maze test in both GH and SI mice (Fig. 4a and b). Furthermore, CLZ (2.5 mg/kg) completely inhibited biting and tail rattling (Fig. 4c and d) and significantly reduced the time spent in aggressive behavior (Fig. 4e) in SI mice.

### 3.4. Repeated FLX treatment improved SI-induced deficit of social behavior

Repeated FLX treatment significantly improved the SI-induced deficit of social behavior to the level in saline-treated GH mice with-

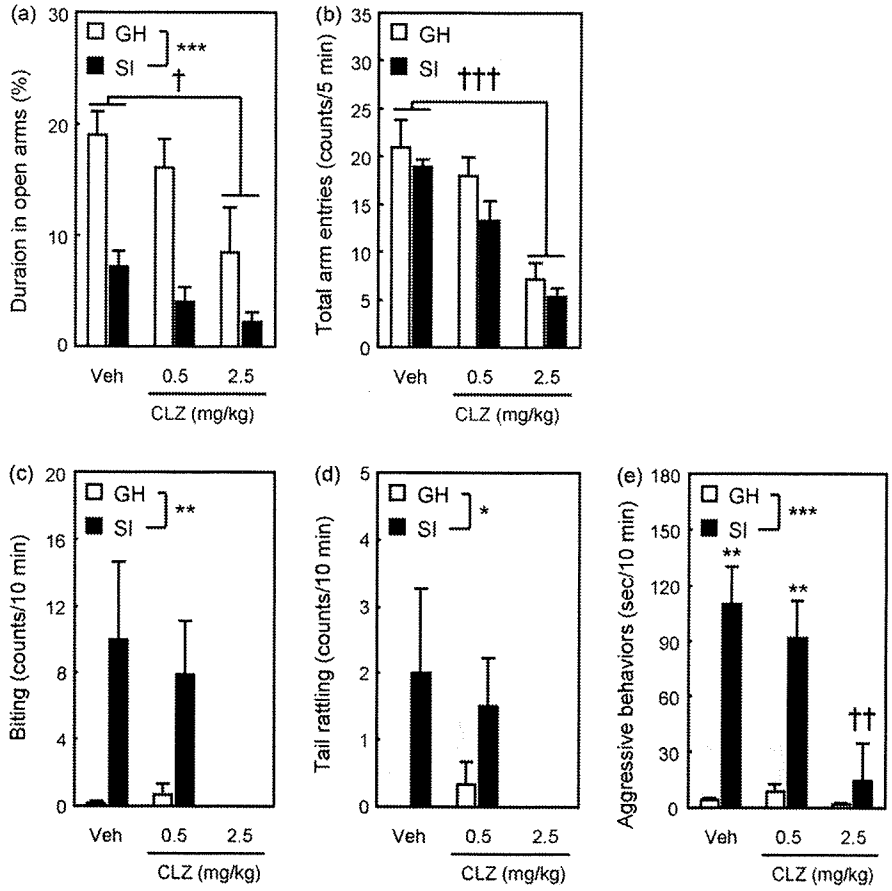


**Fig. 4.** Effect of clozapine (CLZ) on social isolation (SI)-induced anxiety-like and aggressive behavior. CLZ (0.5 or 2.5 mg/kg, i.p.) was administered 30 min before the behavioral test. (a–c) The elevated-plus maze test [vehicle-treated group-housed (GH) mice ($n=6$); CLZ (0.5 mg/kg)-treated GH mice ($n=9$); CLZ (2.5 mg/kg)-treated GH mice ($n=9$); vehicle-treated SI mice ($n=6$); CLZ (0.5 mg/kg)-treated SI mice ($n=9$); CLZ (2.5 mg/kg)-treated SI mice ($n=11$)]. (a) Duration in open arms. Effect of rearing condition: $F(1,44)=25.47$, $p<0.0001$; effect of dose: $F(2,44)=5.155$, $p=0.0097$; interaction: $F(2,44)=1.063$, $p=0.354$. (b) Total arm entries. Effect of rearing condition: $F(1,44)=3.778$, $p=0.058$; effect of dose: $F(2,44)=30.824$, $p<0.0001$; interaction: $F(2,44)=0.404$, $p=0.6702$. (c–e) The intruder-evoked aggressive test [vehicle-treated GH mice ($n=10$); CLZ (0.5 mg/kg)-treated GH mice ($n=9$); CLZ (2.5 mg/kg)-treated GH mice ($n=10$); vehicle-treated SI mice ($n=11$); CLZ (0.5 mg/kg)-treated SI mice ($n=11$); CLZ (2.5 mg/kg)-treated SI mice ($n=11$)]. (c) Frequency of biting. Effect of rearing condition: $F(1,43)=10.187$, $p=0.0026$; effect of dose: $F(1,43)=3.282$, $p=0.0471$; interaction: $F(1,43)=2.757$, $p=0.0747$. (d) Frequency of tail rattling. Effect of rearing condition: $F(1,43)=5.382$, $p=0.0252$; effect of dose: $F(1,43)=2.119$, $p=0.1325$; interaction: $F(1,43)=1.562$, $p=0.2215$. (e) Duration of aggressive behaviors. Effect of rearing condition: $F(1,43)=25.9$, $p<0.0001$; effect of dose: $F(1,43)=5.592$, $p=0.0069$; interaction: $F(1,43)=4.539$, $p=0.0163$. Data are shown as the means ± SE. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. GH mice [ANOVA (a), (c–e) or Bonferroni's *post hoc* test (e)]. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.05$, $^{\dagger\dagger\dagger}p<0.001$ vs. vehicle-treated mice [ANOVA (a)-(b) or Bonferroni's *post hoc* test (e)].

out affecting the social behavior in GH mice (Fig. 5a). Furthermore, repeated FLX treatment significantly reduced aggressive behavior in SI mice (Fig. 5c). There was a tendency for repeated FLX treatment to decrease escape behavior in SI mice, but the effect was not statistically significant (Fig. 5b).

## 4. Discussion

The behavioral, neurochemical and anatomical changes in SI-reared mice may be relevant to the clinical symptoms and pathophysiology in patients with neuropsychiatric disorders, such as ADHD and schizophrenia. To address this issue, we examined the response to drugs used to treat these neuropsychiatric disorders.

First, we investigated the effect of SI rearing after weaning on emotional behavior and recognition memory. SI rearing after weaning induced anxiety-like behavior, aggressive behavior, abnormal social interaction and recognition memory deficits in mice, which is consistent with previous studies reporting anxiety [23,48,51], aggression [29,50], social interaction [12,50] and recognition memory [1,47], respectively. On the other hand, some studies showed an anxiolytic-like effect of SI rearing in the elevated-plus maze test [18,47]. It is possible that SI-induced hyperactivity may be attributable to the anxiolytic-like effect under a novel environment. In our study, SI-reared mice exhibited no difference in locomotor activity and habituation response to a novel environment compared with GH mice. Under such conditions, anxiety-like behaviors were demonstrated with SI rearing.

We found that SI-reared mice were vulnerable to repeated forced swim stress. In contrast, previous reports showed that SI rearing in rodents reduced the immobility time in the forced swim test [19,27]. The discrepancy in the forced swim test may be explained by the difference in behavioral testing. In previous studies, mice were subjected to the forced swim test once or twice, while in the present study, the test was repeated on 5 consecutive days. In fact, there was no apparent difference in immobility time during the first 2 days of the test in the present study.

To assess the predictive validity of SI-reared mice as an animal model of neuropsychiatric disorders, we examined the effects of MPH, CLZ and FLX on SI-induced behavioral abnormalities. We chose the elevated-plus maze test and intruder-evoked aggressive test for MPH and CLZ experiments because anxiety-like behavior and aggressive behavior are the most distinctive alteration of behaviors induced by SI rearing, and ADHD and schizophrenic patients often exhibit anxiety and violence behavior [3,17,25]. The clinical dose range for MPH to treat ADHD is 0.3–0.6 mg/kg (twice

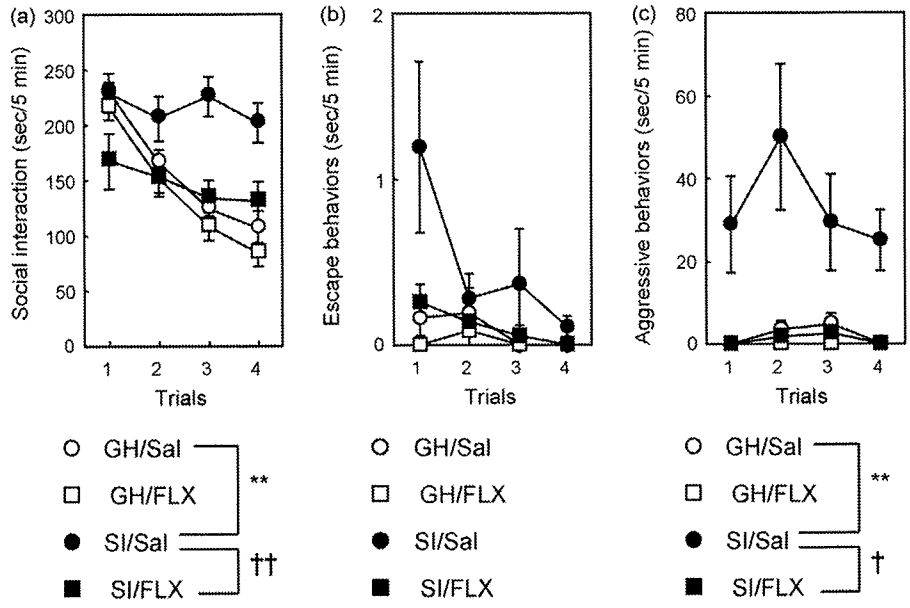


**Fig. 5.** Effect of repeated fluoxetine (FLX) treatment on social isolation (SI)-induced impairment of social behavior. Daily administration of FLX (10 mg/kg, i.p.) was started 2 weeks after SI and continued to the end of the social interaction test. (a) Social behavior. Effect of rearing condition: $F(1,39) = 6.448$, $p = 0.0152$; effect of drug: $F(1,39) = 11.473$, $p = 0.0016$; interaction: $F(1,39) = 4.147$, $p = 0.0485$. (b) Escape behavior. Effect of rearing condition: $F(1,39) = 2.737$, $p = 0.1061$; effect of drug: $F(1,39) = 2.211$, $p = 0.1451$; interaction: $F(1,39) = 1.050$, $p = 0.3119$. (c) Aggressive behavior. Effect of rearing condition: $F(1,39) = 4.741$, $p = 0.0356$; effect of drug: $F(1,39) = 5.191$, $p = 0.0283$; interaction: $F(1,39) = 4.160$, $p = 0.0482$. Data are shown as the means ± SE [GH/Sal: saline-treated group-housed mice ($n = 14$); GH/FLX: FLX-treated GH mice ($n = 7$); SI/Sal: saline-treated SI mice ($n = 14$); SI/FLX: fluoxetine-treated SI mice ($n = 8$)]. $^{**}p < 0.01$ vs. GH mice. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs. saline-treated mice (Bonferroni's *post hoc* test).

a day) and MPH raises resting extracellular levels of dopamine several-fold which in turn inhibits the nerve impulse-associated release [40]. Accordingly, we chose 1–3 mg/kg of MPH in this experiment. Our findings suggested that MPH could improve SI-induced anxiety-like behavior because the drug increased the reduced time spent in open arms in SI mice without affecting the time in GH mice. It is unlikely that the anxiolytic effect arises from hyperlocomotion in MPH-treated mice since total arm entries in both GH and SI mice were not affected by MPH. Previous studies demonstrated that SI rearing in rats altered the time course of locomotor activity induced by amphetamine [26] and increased amphetamine-induced dopamine release in the nucleus accumbens [20]. Therefore, functional changes in dopaminergic systems may be involved in anxiety-like behavior in SI-reared mice as well as the anxiolytic effect of MPH. In the present study, MPH had little effect on SI-induced aggressive behavior, which disagrees with the previous study that MPH inhibited aggression in SI mice [29]. The reason for the discrepancy between studies is unclear at present. Higher doses of MPH may be required to inhibit SI-induced aggressive behavior.

A previous study demonstrated that CLZ at a dose of 2.5 mg/kg reduced aggressive behavior in isolated mice [30]. Consistently, we found that CLZ at same dose markedly suppressed aggressive behavior although it had little effect on anxiety-like behavior in SI-reared mice. However, the anti-aggressive effect of CLZ may be attributed to sedation, because CLZ treatment decreased the number of total arm entries in the elevated-plus maze test. In fact, CLZ at dose of 2.5 mg/kg significantly reduced locomotor activity in both GH and SI mice (data not shown). CLZ has affinities for and antagonizes dopamine (DA) and serotonin (5-HT) receptors. The neurotransmitters DA, 5-HT and gamma-aminobutyric acid have been implicated in the neurobiological mechanisms of aggression [10]. Previous studies showed that the extracellular DA level was increased in the nucleus accumbens during and after aggressive episode in rats [13], while a D1-like antagonist (SCH-23390) or a D2-like antagonist (sulpiride) microinjected into the nucleus accumbens decreased the positive reinforcing properties of aggression in mice [8]. Furthermore, SI rearing induces hyperfunction of the mesolimbic dopaminergic system [15]. Thus, the dopaminergic hyperfunction induced by SI rearing may play a role in the development of aggressive behavior.

The 5-HT system has a role in aggressive behavior [10,36]. Previous studies showed an inverse correlation between the tendency to engage in aggression and a defect of serotonergic neurons in not only rodents [6] but also humans [45]. In accord with this evidence, FLX ameliorated impulsive aggressive behavior in personality-disordered subjects [7] and rodents [14,24]. We have previously reported that a single FLX treatment (10 mg/kg) failed to reverse SI-induced aggressive behavior in the intruder-evoked aggressive test and anxiety-like behavior in the elevated-plus maze, but the aggressive behavior was ameliorated by repeated FLX treatment (10 mg/kg) [24]. Accordingly, in this study social interaction test was conducted to assess the effect of repeated FLX treatment on SI-induced impairment of social behavior. We found that FLX ameliorated the impairment of social behavior as well as aggressive behavior in SI-reared mice. A recent study demonstrated that FLX suppressed the activation of neuronal circuits of aggression during aggressive interaction [14]. The extracellular 5-HT level is decreased in the prefrontal cortex (PFC) during and following aggressive conflict in rats [13]. The 5-$HT_{1A}$ or 5$HT_{1B}$ receptor agonist microinjected in the PFC decreases aggression [5,11]. On the other hand, SI rearing induces the dysfunction of serotonergic neurons; for example, 5-$HT_{1A}$ receptor binding is significantly reduced in the PFC of SI-reared rodents [37,39]. Brain dialysis revealed that 5-HT release induced by KCl in the frontal cortex was attenuated in SI rats [2]. Hence, aggression in SI-reared mice may be attributed to the hypofunction of serotonergic neurons in the brain, especially in the PFC.

In the present study, we showed that long-term SI rearing after weaning affected adult behavior in mice. SI-reared mice exhibited increased vulnerability to swim stress, increased anxiety and impulsivity/aggression, and impaired recognition memory and social interaction. MPH improved SI-induced anxiety

behavior, CLZ reduced SI-induced aggressive behavior, and repeated FLX treatment ameliorated SI-induced abnormality of social behavior. These results suggest that SI-induced behavioral abnormality is a psychobehavioral complex relevant to various clinical symptoms observed in neuropsychiatric disorders, including ADHD, schizophrenia and depression, and that SI-reared mice are valuable to analyze the pathophysiology/pathogenesis of these diseases.

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## References

[1] Bianchi M, Fone KF, Azmi N, Heidbreder CA, Hagan JJ, Marsden CA. Isolation rearing induces recognition memory deficits accompanied by cytoskeletal alterations in rat hippocampus. Eur J Neurosci 2006;24:2894–902.
[2] Bickerdike MJ, Wright IK, Marsden CA. Social isolation attenuates rat forebrain 5-HT release induced by KCl stimulation and exposure to a novel environment. Behav Pharmacol 1993;4:231–6.
[3] Braga RJ, Petrides G, Figueira I. Anxiety disorders in schizophrenia. Compr Psychiatry 2004;45:460–8.
[4] Castellanos FX, Tannock R. Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes. Nat Rev Neurosci 2002;3:617–28.
[5] Centenaro LA, Vieira K, Zimmermann N, Miczek KA, Lucion AB, de Almeida RM. Social instigation and aggressive behavior in mice: role of 5-HT(1A) and 5-HT (1B) receptors in the prefrontal cortex. Psychopharmacology (Berl) 2008;201:237–48.
[6] Chiavegatto S, Nelson RJ. Interaction of nitric oxide and serotonin in aggressive behavior. Horm Behav 2003;44:233–41.
[7] Coccaro EF, Kavoussi RJ, Fluoxetine. impulsive aggressive behavior in personality-disordered subjects. Arch Gen Psychiatry 1997;54:1081–8.
[8] Couppis MH, Kennedy CH. The rewarding effect of aggression is reduced by nucleus accumbens dopamine receptor antagonism in mice. Psychopharmacology (Berl) 2008;197:449–56.
[9] Day-Wilson KM, Jones DN, Southam E, Cilia J, Totterdell S. Medial prefrontal cortex volume loss in rats with isolation rearing-induced deficits in prepulse inhibition of acoustic startle. Neuroscience 2006;141:1113–21.
[10] de Almeida RM, Ferrari PF, Parmigiani S, Miczek KA. Escalated aggressive behavior: dopamine, serotonin and GABA. Eur J Pharmacol 2005;526:51–64.
[11] de Almeida RM, Rosa MM, Santos DM, Saft DM, Benini Q, Miczek KA. 5-HT(1B) receptors, ventral orbitofrontal cortex, and aggressive behavior in mice. Psychopharmacology (Berl) 2006;185:441–50.
[12] Ferdman N, Murmu RP, Bock J, Braun K, Leshem M. Weaning age, social isolation, and gender, interact to determine adult explorative and social behavior, and dendritic and spine morphology in prefrontal cortex of rats. Behav Brain Res 2007;180:174–82.
[13] Ferrari PF, van Erp AM, Tornatzky W, Miczek KA. Accumbal dopamine and serotonin in anticipation of the next aggressive episode in rats. Eur J Neurosci 2003;17:371–8.
[14] Ferris CF, Stolberg T, Kulkarni P, Murugavel M, Blanchard R, Blanchard DC, et al. Imaging the neural circuitry and chemical control of aggressive motivation. BMC Neurosci 2008;9:111–6.
[15] Fone KC, Porkess MV. Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. Neurosci Biobehav Rev 2008;32:1087–102.
[16] Frau R, Orrù M, Fà M, Casti A, Manunta M, Fais N, et al. Effects of topiramate on the prepulse inhibition of the acoustic startle in rats. Neuropsychopharmacology 2007;32:320–31.
[17] Glazer WM, Dickson RA. Clozapine reduces violence and persistent aggression in schizophrenia. J Clin Psychiatry 1998;59:8–14.
[18] Guo M, Wu CF, Liu W, Yang JY, Chen D. Sex difference in psychological behavior changes induced by long-term social isolation in mice. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:115–21.
[19] Hall FS, Huang S, Fong GF, Pert A. The effects of social isolation on the forced swim test in Fawn hooded and Wistar rats. J Neurosci Methods 1998;79:47–51.
[20] Hall FS, Wilkinson LS, Humby T, Inglis W, Kendall DA, Marsden CA, et al. Isolation rearing in rats: pre- and postsynaptic changes in striatal dopaminergic systems. Pharmacol Biochem Behav 1998;59:859–72.
[21] Heidbreder CA, Weiss IC, Domeney AM, Pryce C, Homberg J, Hedou G, et al. Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. Neuroscience 2000;100:749–68.
[22] Heim C, Plotsky PM, Nemeroff CB. Importance of studying the contributions of early adverse experience to neurobiological findings in depression. Neuropsychopharmacology 2004;29:641–8.
[23] Hellemans KG, Benge LC, Olmstead MC. Adolescent enrichment partially reverses the social isolation syndrome. Brain Res Dev Brain Res 2004;150:103–15.
[24] Ibi D, Takuma K, Koike H, Mizoguchi H, Tsuritani K, Kuwahara Y, et al. Social isolation rearing-induced impairment of the hippocampal neurogenesis is associated with deficits in spatial memory and emotion-related behaviors in juvenile mice. J Neurochem 2008;105:921–32.
[25] Jensen PS, Hinshaw SP, Kraemer HC, Lenora N, Newcorn JH, Abikoff HB, et al. ADHD comorbidity findings from the MTA study: comparing comorbid subgroups. J Am Acad Child Adolesc Psychiatry 2001;40:147–58.
[26] Jones GH, Hernandez TD, Kendall DA, Marsden CA, Robbins TW. Dopaminergic and serotonergic function following isolation rearing in rats: study of behavioural responses and postmortem and in vivo neurochemistry. Pharmacol Biochem Behav 1992;43:17–35.
[27] Karolewicz B, Paul IA. Group housing of mice increases immobility and antidepressant sensitivity in the forced swim and tail suspension tests. Eur J Pharmacol 2001;415:197–201.
[28] Lu L, Bao G, Chen H, Xia P, Fan X, Zhang J, et al. Modification of hippocampal neurogenesis and neuroplasticity by social environments. Exp Neurol 2003;183:600–9.
[29] Miczek KA, O'Donnell JM. Intruder-evoked aggression in isolated and nonisolated mice: effects of psychomotor stimulants and L-dopa. Psychopharmacology (Berl) 1978;57:47–55.
[30] Millan MJ, Brocco M, Gobert A, Schreiber R, Dekeyne A. S-16924 [(R)-2-[1-[2-(2,3-dihydro-benzo[1,4]dioxin-5-yloxy)-ethyl]-pyrrolidin-3yl]-1-(4-fluorophenyl)-ethanone], a novel, potential antipsychotic with marked serotonin1A agonist properties: III. Anxiolytic actions in comparison with clozapine and haloperidol. J Pharmacol Exp Ther 1999;288:1002–14.
[31] Mirescu C, Peters JD, Gould E. Early life experience alters response of adult neurogenesis to stress. Nat Neurosci 2004;7:841–6.
[32] Miyamoto Y, Yamada K, Noda Y, Mori H, Mishina M, Nabeshima T. Lower sensitivity to stress and altered monoaminergic neuronal function in mice lacking the NMDA receptor epsilon 4 subunit. J Neurosci 2002;22:2335–42.
[33] Muchimapura S, Mason R, Marsden CA. Effect of isolation rearing on pre- and post-synaptic serotonergic function in the rat dorsal hippocampus. Synapse 2003;47:209–17.
[34] Murphy DA, Pelham WE, Lang AR. Aggression in boys with attention deficit-hyperactivity disorder: methylphenidate effects on naturalistically observed aggression, response to provocation, and social information processing. J Abnorm Child Psychol 1992;20:451–66.
[35] Nagai T, Takuma K, Kamei H, Ito Y, Nakamichi N, Ibi D, et al. Dopamine D1 receptors regulate protein synthesis-dependent long-term recognition memory via extracellular signal-regulated kinase 1/2 in the prefrontal cortex. Learn Mem 2007;14:117–25.
[36] Nelson RJ, Trainor BC. Neural mechanisms of aggression. Nat Rev Neurosci 2007;8:536–46.
[37] Preece MA, Dalley JW, Theobald DE, Robbins TW, Reynolds GP. Region specific changes in forebrain 5-hydroxytryptamine1A and 5-hydroxytryptamine2A receptors in isolation-reared rats: an in vitro autoradiography study. Neuroscience 2004;123:725–32.
[38] Rapoport JL, Addington AM, Frangou S, Psych MR. The neurodevelopmental model of schizophrenia: update 2005. Mol Psychiatry 2005;10:434–49.
[39] Schiller L, Jähkel M, Kretzschmar M, Brust P, Oehler J. Autoradiographic analyses of 5-HT1A and 5-HT2A receptors after social isolation in mice. Brain Res 2003;980:169–78.
[40] Seeman P, Madras B. Methylphenidate elevates resting dopamine which lowers the impulse-triggered release of dopamine: a hypothesis. Behav. Brain Res 2002;130:79–83.
[41] Silva-Gómez AB, Rojas D, Juárez I, Flores G. Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. Brain Res 2003;983:128–36.
[42] Spivak B, Roitman S, Vered Y, Mester R, Graff E, Talmon Y, et al. Diminished suicidal and aggressive behavior, high plasma norepinephrine levels, and serum triglyceride levels in chronic neuroleptic-resistant schizophrenic patients maintained on clozapine. Clin Neuropharmacol 1998;21:245–50.
[43] Sunohara GA, Malone MA, Rovet J, Humphries T, Roberts W, Taylor MJ. Effect of methylphenidate on attention in children with attention deficit hyperactivity disorder (ADHD): ERP evidence. Neuropsychopharmacology 1999;21:218–28.
[44] Tremolizzo L, Doueiri MS, Dong E, Grayson DR, Davis J, Pinna G, et al. Valproate corrects the schizophrenia-like epigenetic behavioral modifications induced by methionine in mice. Biol Psychiatry 2005;57:500–9.
[45] Unis AS, Cook EH, Vincent JG, Gjerde DK, Perry BD, Mason C, et al. Platelet serotonin measures in adolescents with conduct disorder. Biol Psychiatry 1997;42:553–9.
[46] Varty GB, Marsden CA, Higgins GA. Reduced synaptophysin immunoreactivity in the dentate gyrus of prepulse inhibition-impaired isolation-reared rats. Brain Res 1999;824:197–203.
[47] Võikar V, Polus A, Vasar E, Rauvala H. Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences. Genes Brain Behav 2005;4:240–52.

[48] Wei XY, Yang JY, Dong YX, Wu CF. Anxiolytic-like effects of oleamide in group-housed and socially isolated mice. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:1189–95.
[49] Wilkinson LS, Killcross SS, Humby T, Hall FS, Geyer MA, Robbins TW. Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition. Neuropsychopharmacology 1994;10:61–72.
[50] Wongwitdecha N, Marsden CA. Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. Behav Brain Res 1996;75:27–32.
[51] Wright IK, Upton N, Marsden CA. Resocialisation of isolation-reared rats does not alter their anxiogenic profile on the elevated X-maze model of anxiety. Physiol Behav 1991;50:1129–32.

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Research report

# Combined effect of neonatal immune activation and mutant DISC1 on phenotypic changes in adulthood

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ABSTRACT

Gene–environment interaction may play a role in the etiology of schizophrenia. Transgenic mice expressing dominant-negative DISC1 (DN-DISC1 mice) show some histological and behavioral endophenotypes relevant to schizophrenia. Viral infection during neurodevelopment provides a major environmental risk for schizophrenia. Neonatal injection of polyriboinosinic-polyribocytidylic acid (polyI:C), which mimics innate immune responses elicited by viral infection, leads to schizophrenia-like behavioral alteration in mice after puberty. To study how gene-environmental interaction during neurodevelopment results in phenotypic changes in adulthood, we treated DN-DISC1 mice or wild-type littermates with injection of polyI:C during the neonatal stage, according to the published method, respectively, and the behavioral and histological phenotypes were examined in adulthood. We demonstrated that neonatal polyI:C treatment in DN-DISC1 mice resulted in the deficits of short-term, object recognition, and hippocampus-dependent fear memories after puberty, although polyI:C treatment by itself had smaller influences on wild-type mice. Furthermore, polyI:C-treated DN-DISC1 mice exhibited signs of impairment of social recognition and interaction, and augmented susceptibility to MK-801-induced hyperactivity as compared with vehicle-treated wild-type mice. Of most importance, additive effects of polyI:C and DN-DISC1 were observed by a marked decrease in parvalbumin-positive interneurons in the medial prefrontal cortex. These results suggest that combined effect of neonatal polyI:C treatment and DN-DISC1 affects some behavioral and histological phenotypes in adulthood.



## 1. Introduction

Genetic susceptibility factors for schizophrenia have recently become available; these include *neuregulin-1*, *dysbindin*, and *disrupted-in-schizophrenia 1* (*DISC1*) [9]. Maternal viral infection in the first and second trimesters of pregnancy in humans increases the risk of schizophrenia in young adulthood [3,4,22]. Furthermore, the possible interaction between environmental and genetic susceptibility factors, especially during neurodevelopment, is proposed as a promising disease etiology of schizophrenia [5,14].

Here we study a possible interaction of genetic and environmental factors by injecting a synthetic double-stranded RNA, polyriboinosinic-polyribocytidylic acid (polyI:C) into transgenic mice that express a dominant-negative form of DISC1 (DN-DISC1). We chose DISC1 as a genetic factor on which to focus, because its role during neurodevelopment is well characterized [6,13]. DN-DISC1 mice show some behavioral (sensorimotor gating deficits, depression-like behavior and hyperactivity) and histological (enlarged lateral ventricles and reduction in the immunoreactivity of parvalbumin in the cortex) endophenotypes relevant to schizophrenia [10]. PolyI:C is a toll-like receptor 3 ligand that induces a strong innate immune response, and has been used to mimic viral infection during neurodevelopment [15,23].

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